Presented by
Harvard Univ. Press

Discarded by
Dartmouth College Library

Digitized by the Internet Archive
in 2026

https://archive.org/details/reportoffirstexp0000unse

HARVARD SCHOOL OF TROPICAL MEDICINE

REPORT OF FIRST EXPEDITION TO SOUTH AMERICA 1913

MEMBERS OF THE EXPEDITION

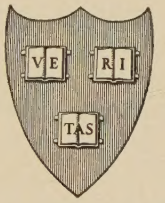
RICHARD P. STRONG
PROFESSOR OF TROPICAL MEDICINE, HARVARD UNIVERSITY MEDICAL SCHOOL

ERNEST E. TYZZER
ASSISTANT PROFESSOR OF PATHOLOGY HARVARD UNIVERSITY MEDICAL SCHOOL

CHARLES T. BRUES
ASSISTANT PROFESSOR OF ECONOMIC ENTOMOLOGY, HARVARD UNIVERSITY

A. W. SELLARDS
ASSOCIATE IN TROPICAL MEDICINE HARVARD UNIVERSITY MEDICAL SCHOOL

J. C. GASTIABURU
DIRECTOR MUNICIPAL LABORATORY OF HYGIENE, LIMA



CAMBRIDGE
HARVARD UNIVERSITY PRESS
1915

Discarded by
Dartmouth College Library

Gift of
Harvard University Press.

616.92
q H261r

COPYRIGHT, 1915
HARVARD UNIVERSITY PRESS

D 18'17

140316

Harvard univ. Medical school. Dept. of tropical medicine.

PLYMOUTH
COLLEGE
LIBRARY

TABLE OF CONTENTS

I. INTRODUCTION

II. GENERAL CONSIDERATION OF OROYA FEVER AND OF VERRUGA PERUVIANA

PAGE

Historical review 8
Previous views regarding etiology 10
Generally accepted opinion regarding the nature of verruga peruviana 13
Present investigations 14

III. OROYA FEVER

Distribution and seasonal prevalence 15
Clinical features 17
Blood 19
Cases emphasizing special clinical features 21
Pernicious Oroya fever terminating in death 21
Mild case of Oroya fever complicated with verruga peruviana . . 24
Moderately severe Oroya fever terminating in recovery 25
Description and classification of parasite of Oroya fever 28
Forms observed in fresh blood preparations 28
Stained and fixed preparations of blood 29
Classification 30
Development within endothelial cells 32
Attempts to infect animals with the parasite of Oroya fever 34
Experiments 35
Conclusions 37
Experiments in the cultivation of microörganisms from blood of Oroya fever cases 37
Relationship to paratyphoid infection 39
Attempts to infect and observe development of *Bartonella bacilliformis* in mosquitoes 41
Pathology 42
Previous investigations. 42
Present investigations 44
Histopathology 49
Present investigations 49
Studies of various tissues for diagnosis 53

IV. VERRUGA PERUVIANA

PAGE

Geographical distribution 57
Conditions in the endemic areas 58
Etiology 61
Clinical features 64
Incubation period 64
Symptoms 64
Eruption 65
Blood 67
Prognosis 69
Cases illustrating special features of the disease 69
Case 1. Extensive eruption; miliary, nodular and mulaire lesions 69
Case 17. Papular, cutaneous, and subcutaneous nodular eruption 73
Case 19. Extensive nodular and mulaire lesions 75
Case 2. Showing only miliary eruption 76
Cases 4 and 5. Course of the disease in children 78
Case 9. Eruption upon palms of hands and soles of feet . . 79
Case 13. Single mulaire lesion 80
Case 14. Verruga complicated with malaria 81
Experiments relating to the nature of the virus of verruga peruviana 81
Transmission to animals, cultivation, filterability 81
Experiments with Case 1 82
Experiments with Case 16 105
Experiments with Case 7 107
Experiments with Case 17 110
Experiments with Case 19 114
Experiments with Case 21 119
Experiments with Case 25 121
Summary of transmission experiments to animals 122
Cultivation and filterability of the virus of verruga 125
Similarity between the virus of verruga and that of small-pox . . 129
Differential diagnosis of verruga peruviana 130
From framboesia 130
From Oroya fever 133
Relationship to paratyphoid fever. 135
Relationship to angiofibroma cutis circumscriptum contagiosum . 136
Pathology 137
Gross pathology 137
Histology 140
Previous investigations 140
Present investigations (human and animal lesions) 145
Vaccination and immunity 152
Transmission of verruga peruviana by arthropoda 153

PAGE

V. ENTOMOLOGICAL INVESTIGATIONS AT MATUCANA . . 161

VI. UTA

Clinical description, etiology, and pathology 175

VII. GUAYAQUIL

Sanitary conditions 180
Prevailing diseases 182
Entomological investigations 184

VIII. YELLOW FEVER

Investigation of the blood 189
Clinical histories 190
Pathology 191

IX. LINGUATULIDA FROM CROCODILES 201

X. ACKNOWLEDGMENTS 203

APPENDIX

I. Porocephalus Crocodili 207
II. Some flies of the family Phoridæ 209
III. Notes on Peruvian mosquitoes 212
IV. Heteroptera from the west coast of South America 218
V. List of bees collected by the expedition 220

LIST OF ILLUSTRATIONS

PLATE		PAGE
I.	Buenaventura, Colombia	4
	Fig. 1. General character of dwellings in the town.	
	Fig. 2. General character of dwellings on outskirts of town.	
II.	Chronic Lesions of the Skin Observed in South America	4
	Fig. 1. Espundia.	
	Fig. 2. Chronic ulcerative process.	
	Fig. 3. Phagedenic ulceration.	
	Fig. 4. Yaws in Buenaventura.	
III.	City of Guayaquil	6
	Fig. 1. Showing elevation above river and open drains.	
	Fig. 2. General view of the city.	
IV.	Oroya Fever	26
	Fig. 1. Parasite of Oroya fever in fresh blood specimen, showing changes in shape and position in the cell that the organism underwent during a period of one and one-half hours in which it was observed.	
	Fig. 2. Parasite of Oroya fever in fresh blood specimen (bacillary and rounded forms).	
V.	Parasite of Oroya Fever in Stained Blood Specimens	28
	Figs. 1 and 2.	
VI.	Oroya Fever	28
	Figs. 1 and 2. Parasite of Oroya fever in stained blood specimen.	
	Fig. 3. Isolated endothelial cell.	
	Fig. 4. Section showing cycle of *Bartonella bacilliformis* in endothelial cells of lymphatic gland.	
VII.	Oroya Fever Parasite in Red Blood-cells (Stained Preparations)	30
	Fig. 1. Mild infection.	
	Figs. 2, 3, 4, 5, 6. Severe infection.	
	Figs. 1, 2, 3, 4, 6, = × 1500. Fig. 5, = × 2000.	
VIII.	Oroya Fever	30
	Fig. 1. Lymph-node. Showing swollen endothelial cells in blood-vessel wall.	
	Fig. 2. Smear from lymph-node. Endothelial cells. Showing phase in multiplication of parasite. Smear stained by Giemsa's method.	

PLATE PAGE

IX. Oroya Fever, Lymph-node 32
Fig. 1. Endothelial cell in blood-vessel of lymph-node. Showing parasite in form of rounded bodies with minute chromatin granules.
Fig. 2. Endothelial cell in blood-vessel of lymph-node. Showing later stage of parasite, i. e., rod forms.

X. Oroya Fever. Endothelial Cells Showing Various Stages in the Development of the Parasite; early Rounded Forms later Breaking up into Rods. . . 34
Fig. 1. An endothelial cell containing rounded bodies with bluish cytoplasm and small granules of chromatin (interpreted as an early stage in the development of the parasite).
Fig. 2. A small endothelial cell containing a number of bluish parasites, each containing several distinct granules of chromatin. At this stage none appear to contain more than four granules. To the right is a red blood-corpuscle containing three rods, each showing a chromatin granule with denser cytoplasm at the opposite pole.
Fig. 3. A large endothelial cell distended with rounded masses, each containing a large number of minute points of chromatin. The bodies are more or less uniform in size, and the chromatin granules are more minute than in the preceding stages.
Fig. 4. An endothelial cell containing a large number of minute elements which appear to be elongated and to contain chromatin granules.
Fig. 5. An endothelial cell showing numerous minute elements which appear distinctly rod-shaped when isolated.
Fig. 6. A mass of rod forms found free from the cell. In the two red blood-corpuscles to the right are characteristic rod-shaped parasites which may serve as a basis of comparison.
Fig. 7. A cell containing small bodies most distinct in the upper left hand extremity, each with a small number of chromatin points representing a stage similar to that in Fig. 2.

XI. Oroya Fever 34
Fig. 1. Life cycle of *Lympho-haematocytozoön parvum* (after Gonder).
Fig. 2. Ulceration in large intestine.

XII. Oroya Fever 48
Fig. 1. Spleen. Area of necrosis at border of malpighian nodule.
Fig. 2. Liver. Showing area of necrosis, central type.

PLATE PAGE

XIII. Oroya Fever 48
Fig. 1. Liver. Stained with scharlach Roth. Showing areas of central necrosis.
Fig. 2. Liver. Showing area of necrosis. Fatty degeneration of liver cells and phagocytosis.

XIV. Oroya Fever Liver 50
Fig. 1. Showing necrosis and the character of pigment.
Fig. 2. Showing endothelial phagocytes in sinusoids, enclosing erythrocytes, erythroblasts, polymorphonuclear leukocytes, and pigment.

XV. Oroya Fever Spleen showing Fibrin Deposition and Character of Pigment 50

XVI. Oroya Fever 52
Fig. 1. Liver. Showing endothelial phagocytes in sinusoid.
Fig. 2. Lymph-node. Showing swollen endothelial cell in blood-vessel wall.

XVII. Peru 58
Fig. 1. Regions in the Andes Mountains in which Oroya fever, verruga peruviana, and malaria are prevalent.
Fig. 2. View down the Rio Rimac from above Matucana, showing the town.

XVIII. Peru 58
Fig. 1. Type of vegetation on the lower slopes of the Cañon close to the river along the trail from Surco to Matucana.
Fig. 2. Type of vegetation covering the higher slopes of the Cañon along the trail from Surco to Matucana.
Fig. 3. Oroya Fever Bridge in the Andes Mountains.

XIX. Verruga Peruviana (From Lumiere Plates) 64
Fig. 1. Early miliary eruption.
Fig. 2. Miliary eruption. More advanced stage.
Fig. 3. Eruption. Most active stage of disease.
Fig. 4. Eruption. Beginning to recede.

XX. Verruga Peruviana (From Lumiere Plates) 64
Fig. 1. Nodular, subcutaneous, and mulaire lesions.
Fig. 2. Nodular and mulaire lesions.

XXI. Verruga Peruviana 66
Fig. 1. Acute stage of disease.
Fig. 2. Advanced mulaire lesion.

XXII. Verruga Peruviana 66
Fig. 1. Showing nodular and subcutaneous lesions.
Fig. 2. Showing nodular lesions.

XXIII. Verruga Peruviana. Showing General Distribution of Nodular Lesions 68

XXIV. Verruga Inoculata in the Monkey 122

PLATE PAGE

XXV. VERRUGA PERUVIANA. EXPERIMENTAL LESIONS PRODUCED IN MONKEYS BY INOCULATION OF HUMAN VIRUS 122

XXVI. VERRUGA PERUVIANA 124
Fig. 1. Dog's testis. Showing experimental lesion produced by the inoculation of human virus.
Fig. 2. Rabbit's testis. Showing lesion produced by the inoculation of human virus.

XXVII. VERRUGA PERUVIANA 144
Fig. 1. Section of skin lesion. Showing proliferation of angioblasts about vessels.
Fig. 2. Very early skin lesion. Showing endothelial proliferation about vessels.

XXVIII. VERRUGA PERUVIANA 144
Fig. 1. Early skin lesion. Showing very vascular structure.
Fig. 2. Skin lesion. Section stained with phosphotungstic acid stain to show vascularity.

XXIX. VERRUGA PERUVIANA 146
Fig. 1. Nodule of skin. Showing islands of closely-placed angioblasts, oedematous connective tissue, and numerous vessels.
Fig. 2. Nodule of skin. Showing islands of angioblasts, oedematous connective tissue, and numerous vessels.

XXX. VERRUGA PERUVIANA 146
Fig. 1. Section of nodule showing particularly mitosis.
Fig. 2. Section showing proliferation of angioblasts, and appearance of fibroblasts.

XXXI. VERRUGA PERUVIANA. LESIONS OF THE SKIN 148
Fig. 1. Section stained with eosin and methylene blue. × 10.
Fig. 2. Section stained with Mallory's connective tissue stain. × 10.

XXXII. VERRUGA PERUVIANA. LESIONS OF THE SKIN 148
Fig. 1. Section stained to show connective tissue.
Fig. 2. Section showing cellular structure.

XXXIII. 164
Fig. 1. *Phalangomyia debilis* Dyar and Knab. Full-grown larva, dorsal view.
Fig. 2. *Phalangomyia debilis* Dyar and Knab. Pupa, lateral view.
Fig. 3. *Aphiochaeta scalaris* Lw. Lateral view of male.

XXXIV. 168
Fig. 1. Culicine larva. Full-grown, dorsal view. Surco.
Fig. 2. Simulium sp. Full-grown larva. Matucana.

XXXV. UTA 174
Fig. 1. Early papule above right lip. Large numbers of Leishmania found.
Fig. 2. Slightly more advanced lesion on lip than No. 1.

PLATE PAGE

XXXVI. Uta 174
Fig. 1. Early lesions on mother and child, slightly more advanced than shown in Plate XXXV, Fig. 2.
Fig. 2. Very advanced lesions.

XXXVII. Uta (From Lumiere Plates). Healing Stage of an Advanced Case 176

XXXVIII. Uta. Preparation made from a Lesion in the Early Stage of the Disease, showing Species of Leishmania, the Etiological Factor 178
Figs. 1 and 2.

XXXIX. Uta 178
Fig. 1. Preparation made from a lesion in the early stage of the disease, showing species of Leishmania, the etiological factor.
Fig. 2. Flagellate forms of Leishmania obtained in culture from an Uta case.

XL. Uta 178
Fig. 1. Papular lesions on inner aspect of dog's ears. Appearing about one month after inoculation with material from early case of Uta.
Fig. 2. Showing Leishmania in a section of the lesion.

XLI. Guayaquil 182
Fig. 1. Plague Hospital.
Fig. 2. Yellow Fever Hospital.

XLII. Guayaquil 182
Fig. 1. Homes of yellow fever immunes.
Fig. 2. Open drain. Breeding place of Mosquitoes.

XLIII. Guayaquil 184
Fig. 1. Open drain behind the Yellow Fever Hospital.
Fig. 2. Breeding places of yellow fever and malaria mosquitoes.

XLIV. 184
Fig. 1. Milagro, near Guayaquil. A yellow fever district.
Fig. 2. Surco, Peru, where uta, Oroya fever, malaria, and verruga peruviana are prevalent.

XLV. Section of Bubo in Plague 186

XLVI. Liver in Yellow Fever. 189

XLVII. Ecuador 202
Fig. 1. Crocodile (*Crocodilus americanus*) shot in the Guayas river.
Fig. 2. Daule river. Favorite haunt of crocodiles.

XLVIII. Linguatulida from Lung of Crocodile 202
Fig. 1. Adult Linguatulida. About × 21.
Fig. 2. Cross-section of adult. × 114.
Fig. 3. Greater magnification of head. Showing mouth and hooklets. About × 61.

PLATE PAGE

TEXT FIGURES:

Fig. 1. *Phalangomyia debilis.* Antenna of larva 165
Fig. 2. *Phalangomyia debilis.* Mandible of larva 165
Fig. 3. *Phalangomyia debilis.* Maxilla 165
Fig. 4. *Phalangomyia debilis.* Mental plate of larva . . . 165
Fig. 5. *Phalangomyia debilis.* Posterior portion of larva . 166
Fig. 6. Simulium larva, No. 1. Labium 168
Fig. 7. Simulium larva, No. 2. Labium 168
Fig. 8. Simulium pupa, No. 3 169
Fig. 9. Simulium pupa, No. 4 170

CLINICAL CHART 20

TABLES I AND II 122

THE HARVARD SCHOOL OF TROPICAL MEDICINE

REPORT OF THE FIRST EXPEDITION TO SOUTH AMERICA, 1913

I

INTRODUCTION

The first expedition from the Harvard School of Tropical Medicine was undertaken for the purpose of investigating certain forms of tropical disease in South America, particularly verruga peruviana, as well as with the idea of collecting material to be used for the instruction of students in the various courses of the School, opened in 1913.

The expedition proceeded first to Kingston, Jamaica, and from there to Colon and Panama. Observations were made concerning the prevailing diseases in these localities and considerable pathological material was collected. In Kingston, malaria, uncinariasis, amoebic and bacillary dysentery, and filariasis are common diseases. An obscure condition known as "vomiting sickness" has been reported as occurring in Jamaica for many years. Since 1904 it has been more widely discussed in medical literature. More recently the investigations of Scott and Seidelin have attracted attention to it. It would appear that while microörganisms have been found associated with this disease, its etiology is still not definitely determined. Further observations regarding this condition are desirable.

The sanitary conditions in the Canal Zone and the diseases prevailing there have been already widely discussed elsewhere, and therefore no account of our observations made in these localities will be given in this report. The wards of the Panama City hospital contain many patients afflicted with various tropical diseases, and in the government hospital at Ancon a large number of individuals suffering with malaria may be found.

The voyage was next continued down the west coast of South America to Buenaventura, Colombia. This city is situated upon an island at the mouth of the San Juan river in

the region of mangrove swamps. At ebb tide extensive mud flats extend out from the banks of the river on which the town is built. There are no docks and the ships anchor at some distance from the shore. The town is said to have a population of 10,000 at the present time, the great majority of which are negroes. The town has no system of drainage and the back yards of the houses are filthy and filled with rubbish. Mosquitoes and other insects are very numerous about the outskirts of the town and in the mangrove swamps. A large proportion of the inhabitants suffer with skin diseases of which caraate is by far the most common. Individuals with leprosy, yaws, and chronic ulcerative processes of the skin are also frequently observed. (Plates I and II.) Pathological and entomological material was also collected here.

From Buenaventura we proceeded directly to Guayaquil, Ecuador. From a sanitary standpoint Guayaquil is a city of considerable importance to a number of civilized countries. It is the largest port of Ecuador and is the last port of call of many steamers on the northward route to Balboa and the Panama Canal. (Plate III.) At the present time it is one of the most unsanitary cities in the world. It is particularly dangerous for visitors on account of the risk to which they are exposed of contracting yellow fever. The city is situated two and one-half degrees south of the equator, on the right bank of the Guayas river, about forty miles from its mouth. The population is estimated at 80,000. The prevailing diseases are yellow fever, malaria, bubonic plague, dysentery, hookworm infection, and typhoid fever. Cases of yellow fever occurred almost daily during our stay in Guayaquil, and we had opportunities of examining a large amount of clinical material and of obtaining at autopsy abundant and excellently preserved material for histological study. The blood in yellow fever was studied with relation to the presence of parasites, particularly with reference to the existence of the protozoön which Seidelin has described under the name of *Paraplasma flavigenum* as the cause of the disease. We were unable, however, to detect any bodies which suggested a parasitic nature in the

Fig. 1. — General Character of Dwellings in the Town.

Fig. 2. — General Character of Dwellings on Outskirts of Town.

Plate I. — Buenaventura, Colombia.

Fig. 1. — Espundia.

Fig. 2. — Chronic Ulcerative Process.

Fig. 3. — Phagedenic Ulceration.

Fig. 4. — Yaws in Buenaventura.

Plate II. — Chronic lesions of the skin observed in South America.

blood in this disease. Cultures were also made from the blood by the various modern methods, but no visible causative organism could be detected. A more detailed account of the city of Guayaquil will be found in the section relating to the sanitary conditions of this city and the entomological investigations carried on there. The studies relating to yellow fever are also discussed in Section VIII of this report.

In the study of the animal parasites harbored by the crocodiles which abound upon the banks of the rivers near Guayaquil, a species of Linguatulida was discovered. A description of this parasite is found on page 207.

From Guayaquil we proceeded by boat to Callao, and thence by rail to Lima, Peru. In the latter city and in a number of mountain towns in the interior of Peru a large amount of the work of the expedition was carried on. The diseases particularly investigated in these localities were verruga peruviana, Oroya fever, and uta. Isolation by mountain ranges together with the unusual climatic conditions which prevail in certain portions of Peru, situated as they are, so near to the equator, undoubtedly have exercised a remarkable influence upon producing what is in some respects at least a very unusual fauna and flora, and hence it perhaps is not surprising that unusual diseases should be encountered in these regions.

From remote historical times the inhabitants of Peru have suffered severely from a disease known as verruga peruviana, which is characterized by fever, anaemia, and a nodular eruption on the skin. Hitherto it has been the generally accepted opinion that this disease in its severe form was preceded by an initial stage known as the Oroya fever stage, or the *fiebre grave de Carrion*. If the individual did not die in this febrile stage the fever abated and then the eruptive or verruga stage of the disease was said to begin.

The cause of the malady was hitherto not definitely known, but the majority of investigators have attributed a bacterial origin to it. Barton described a bacillus as the cause, and later observed bodies in the red cells which he believed to be protozoa; by other observers these were usually thought to

be products of cell degeneration. More recently a species of Leishmania or one of Chlamydozoa has been regarded as the cause of the disease. From our investigations which are recorded in detail in this report, it would appear that verruga peruviana and Oroya fever are two distinct diseases; that the former is due to a virus which may be transmitted easily to animals, particularly monkeys, producing characteristic lesions in them, while Oroya fever owes its origin to a protozoan parasite of the red blood-corpuscles and endothelial cells, sufficiently distinct from other protozoa previously described to be placed in a new genus. To this parasite we have given the name of *Bartonella bacilliformis*. Also we have been able to demonstrate a method of vaccination against verruga peruviana. A detailed description of our observations relating to these diseases will be found in Sections III and IV.

Uta is an ulcerative disease which has been known in Peru since prehistoric times. Lesions of the malady have been depicted on the "*huacos*" or ancient pottery of the Incas. It has been supposed that the disease was a prehistoric form of syphilis or one of leprosy. More recently it has been regarded as a form of lupus vulgaris, or as a distinct infection. Its etiology hitherto had not been determined. We were able to show that uta is due to a species of Leishmania. We were also able to successfully obtain the flagellate stage of the organism in cultures and to inoculate successfully a dog with it. The researches undertaken in connection with uta will be found in Section VI.

Acknowledgment of both official and financial assistance rendered the expedition will be found at the end of the report. The other scientific members of the expedition were Doctor E. E. Tyzzer, Assistant Professor of Pathology, C. T. Brues, Assistant Professor of Economic Entomology, both of Harvard University, and Dr. A. W. Sellards, formerly of Johns Hopkins University and now Associate in Tropical Medicine at Harvard University. In Lima the services of Dr. J. C. Gastiaburú, Chief of the Institute of Hygiene, Lima, were enlisted in connection with the work in Peru. Miss Nora A. Dwyer,

FIG. 1. — SHOWING ELEVATION ABOVE RIVER AND OPEN DRAINS.

FIG. 2. — GENERAL VIEW OF THE CITY.

PLATE III. — CITY OF GUAYAQUIL.

Secretary to President A. Lawrence Lowell, accompanied the expedition as secretary.

The results obtained and the discoveries made by the expedition should serve to emphasize the importance of sending from the School to the tropics other expeditions of this nature for the investigation of specific problems. In this instance the additional knowledge obtained relating to the diseases studied has been secured at a comparatively trifling cost.

II

GENERAL CONSIDERATION OF OROYA FEVER AND VERRUGA PERUVIANA

Historical Review

From remote historical times the inhabitants of Peru are said to have suffered severely from an obscure disease characterized by fever, anaemia, and a nodular eruption upon the skin. Over four centuries ago, during the reign of the Inca, Huayna Capac, thousands of lives were swept away, supposedly from this malady, and in the history of the conquest of Peru by Zarate,[1] published in 1545, it is stated that the disease is more destructive than small-pox, and almost as disastrous as the plague itself. Later this author mentions that the Portuguese soldiers were afflicted by boils or warts of a very malignant kind, and that not a single person in the army escaped them. De la Vega also relates that during the 16th century a quarter of the invading army of Peru under Francisco Pizarro perished from this disease. In 1842 Archibald Smith [2] called attention again to the malady, and shortly afterward a number of additional papers relating to it were published by Tschudi,[3] Odriozola,[4] and Salazar.[5] Later Dounon [6] in 1871, Fournier [7] in 1874, Bourse [8] and Tupper [9] in 1877, all described the disease.

[1] Zarate: Historia del discubrimento de Peru, 1545, i, 4; ii, 1.
[2] Smith: Edinb. M. and S. J., 1842, 67.
[3] Tschudi: Arch. f. physiol. Heilk., Stuttg., 1845, iv, 378.
Oesterr med. Wchnschr., Wien, 1846, 505.
[4] Odriozola: Gac. méd. de Lima, 1858, April.
Med. Times and Gaz., Lond., 1858, Sept., 280.
[5] Salazar: Thesis, Gac. méd. de Lima, 1860.
[6] Dounon: Arch. de méd. nav., Par., 1871, 255.
Thèse de Paris, 1871.
[7] Fournier: Arch. de méd. nav., Par., 1874, 156.
[8] Bourse: Arch. de méd. nav., Par., 1876, 353.
[9] Tupper: Ueber die Verruca peruviana, Inaug.-Diss., Berl., 1877.

In 1870 a severe outbreak of fever took place among the workmen building the Central Railway between Lima and Oroya, and it is estimated that at least seven thousand individuals died during the epidemic. At this time the complaint received the name of "Oroya fever," although it appears that it was not contracted in the town of Oroya itself. Bourse reported that all of the engineers superintending the building of the Trans-Andean Railway contracted verruga, and that half of them died of it. Of forty sailors who had deserted from a British ship and gone to work on the railway, thirty died of the disease in the course of seven or eight months. In 1906 out of a force of two thousand men employed in tunnel work for the Central Railway, two hundred are known to have died of the disease. It appeared to be only necessary for the workmen to spend a single night in the infected districts in order to contract the malady.

Previous to 1885 there ensued some discussion as to whether Oroya fever and verruga peruviana were related to one another, or whether the latter was a distinct disease. August 27, 1885, Daniel Carrion, a medical student in Lima, and a native of Cerro de Pasco, Peru (a town situated in the mountains far above the localities in which the disease abounds), attempted to solve this problem by vaccinating both his arms with blood from a verruga nodule. It is related that twenty-three days later he began to suffer from Oroya fever, from which he died sixteen days later, or on October 5. From this experiment the conclusion was drawn by Peruvian physicians that verruga and Oroya fever were only different stages of the same disease, and this is the opinion which has been held generally by them since this time. In honor of Carrion's attempt to throw light on the question, the febrile condition, which has been regarded as the first stage of the malady, is now generally known in Peru as "fiebre de Carrion." Although it has been stated that Carrion during his illness kept notes, and gave a minute description of his symptoms to his companions, unfortunately it appears that none of these were preserved and published. No accurate record of Carrion's case and of the

necropsy is available. It has been suggested since that he died of typhoid fever or of a more acute form of septicemia, and it is also quite possible that the patient from which he infected himself was suffering with Oroya fever as well as verruga at the time.

In 1898 the monograph of Ernesto Odriozola [1] appeared, which constitutes the most excellent and most comprehensive account of the disease which has been written. To this monograph the reader is referred for the complete history of the disease, its geographical distribution, and indeed for all points concerning our knowledge of it and its cause up to that date.

Previous Views Regarding Etiology

If we turn our attention to the studies which have been carried on previously in relation to the etiology of these conditions, we find very conflicting opinions, although the majority of writers on this subject have ascribed a bacterial origin to the disease. Izquierdo [2] in 1884 reported the presence of a bacillus in the study of sections of tissues sent him from Peru, and Florez [3] in 1887 from the blood of individuals affected with verruga cultivated on agar-agar a coccus. Odriozola [4] also reported the presence of a bacillus in the blood.

In 1901 and 1902 Barton [5] performed extensive bacteriological investigations, and concluded that in the blood and organs at necropsy of persons dying from Carrion's fever a bacillus was present which, though similar to *Bacillus coli communis*, could be distinguished from it. This organism, which he regarded as the cause of the disease, was said to give rise either to a fatal septicemia in animals, or to a verruga-like eruption of the skin. Biffi and Carbajal,[6] and Tamayo and

[1] Odriozola: La Maladie de Carrion ou la Verruga Péruvienne, Par., 1898, Carré et Naud, Éditeurs.

[2] Izquierdo: Virchow's Arch. f. path. Anat., Berl., 1885, xcix, 411.

[3] Florez: Odriozola's Monograph, p. 100.

[4] Odriozola: loc. cit., p. 102.

[5] Barton: Crón. méd., Lima, 1901, xviii, No. 301, p. 193; No. 302, p. 210; 1902, xix, No. 334, p. 348.

[6] Biffi and Carbajal: Crón. méd., Lima, 1904, xxi, No. 379, p. 285; 1903, xx, No. 346, p. 149. Biffi: Arch. f. Schiffs- u. Tropenhyg., Leipz., 1908, xii, 1.

Gastiaburú [1] investigated this bacillus, and reported that it was present constantly in persons suffering from Carrion's fever, and was agglutinated by their serum, but was absent from persons suffering from verruga peruviana without fever, whose serum also showed no agglutination of this bacillus. They identified the organisms isolated as belonging in the paratyphoid group. They were unable to confirm Barton's results as to the production in animals of a skin eruption by the bacillus, and concluded that it may be a secondary invader in verruga, or may give rise to the symptoms of a form of fever resembling typhoid which constituted the *fiebre grave de Carrion.*[2] In 1903 Biffi [3] and Gastiaburú [4] noted in stained preparations of the blood the presence of granules in the red blood-cells which stained readily with the basic anilin dyes. The form and structure of these bodies was that of a coccobacillus or of irregular granules. In 1905, before the Sociedad Medica Unión Fernandina, Barton [5] described in the red blood-cells of two persons sick with severe malignant fever elements similar in morphology to bacilli. In 1909 he noted the presence of these elements in stained blood specimens in fourteen additional cases and expressed the belief that they were protozoa and probably the specific agent of the infection. In 1909 Gastiaburú and Rebagliati [6] observed the bodies described by Barton and stated that they were probably protozoa and might be regarded as the pathogenic organism in Carrion's disease. Later, De Vecchi,[7] Mayer,[8] Gastiaburú and Rebagliati,[9] Monge,[10] and several other observers have

[1] Tamayo and Gastiaburú: Gac. de l. hosp., 1905, ii, No. 46, p. 516; Crón. méd., Lima, 1905, xxii, No. 406, p. 335; No. 407, p. 349; 1906, xxiii, No. 429, p. 327; 1907, xxiv, No. 453, p. 321; Gac. de l. hosp., 1906, iii, No. 62, p. 107.

[2] Biffi believed that it was not proved that verruga peruviana and the severe fever of Carrion were two stages of one and the same disease.

[3] Biffi: Crón. méd., Lima, 1903, xx, No. 346, p. 149.

[4] Gastiaburú: Crón. méd., Lima, 1903, xx, No. 356, p. 314.

[5] Barton: Crón. méd., Lima, 1909, xxvi, No. 481, p. 7.

[6] Gastiaburú and Rebagliati: Crón. méd., Lima, 1909, xxvi, No. 501, p. 378.

[7] De Vecchi: Beiheft 4, Arch. f. Schiffs- u. Tropenhyg., Leipz., 1909, xiii, 143.

[8] Mayer: Centralbl. f. Bakteriol. u. Parasitenk., Jena, 1910, lvi, Orig. 309.

[9] Gastiaburú and Rebagliati: Crón. méd., Lima, 1912, xxix, No. 571, p. 644; No. 572, p. 651.

[10] Monge: J. Lond. School Trop. Med., 1912, i, Part 2, 163; Crón. méd., Lima, 1912, xxix, No. 571, p. 640.

been inclined to believe that the bodies described by Barton were products of cell degeneration. Bassett-Smith [1] and Galli Valerio [2] studied several stained specimens of the blood sent them from Peru. Bassett-Smith concludes that before any opinion is arrived at as to the nature of the inclusions in the red cells, a detailed study of a large number of fresh specimens should be made to see if their presence is constant in all acute cases. Galli Valerio mentions that in the red cells, he observed the bodies previously described, some of which in one specimen resembled *Anaplasma marginale* of cattle. He regards the etiology of verruga peruviana as still undetermined. On the other hand, Nicolle,[3] Letulle,[4] and Galli Valerio,[5] found acid-fast bacilli in the lesions from patients with verruga. Darling,[6] whose publication relating to this disease is based on a necropsy made in Panama the day following the death of a patient with verruga complicated with tuberculosis, suggests that the acid-fast bacilli previously described were really tubercle bacilli occurring in cases complicated by tuberculosis. In the same paper Darling apparently from an examination of stained blood films not from his own case, but sent him from Peru by Barton, concludes that the slender rod-like bodies in the red cells which he refers to as "x bodies," seemed to represent some unique type of microorganism. In his own case of verruga no "x bodies" were observed in the preparations, with the possible exception of the smears made from the rib marrow, which he states contained a few rod-like bodies, not definitely in the erythrocytes. Still more recently Gastiaburú and Rebagliati [7] have found in the liver and skin lesions of a verruga patient in the eruptive phase, certain bodies, at times endoglobular

[1] Bassett-Smith: Brit. M. J., Lond., 1909, ii, 783.

[2] Valerio, Galli: Centralbl. f. Bakteriol. u. Parasitenk., Jena, 1911, lviii, Part I, Orig., 228.

[3] Nicolle: Ann. de l'Inst. Pasteur, Par., 1898, xii, 591.

[4] Letulle: Compt. rend. Soc. de biol., Par., 1898, 764; Odriozola's Monograph, p. 201.

[5] Valerio, Galli: loc. cit.

[6] Darling, S. T.: J. Am. M. Ass., Chicago, 1911, Dec. 23, lvii, 2071.

[7] Gastiaburú and Rebagliati: Crón. méd., Lima, 1912, xxix, No. 571, p. 644; No. 572, p. 651.

in leukocytes and other cells, and at other times free, which from their staining reactions and morphologic appearance they regarded as probably organisms of the genus Leishmania. Rebagliati also found certain endoglobular bodies which he considered to be remnants of nuclear disorganization of the erythrocytes, which suggested that Barton's bodies might be chromatin filaments, segregated from the nuclei of erythroblasts. Finally, during the past year, Mayer, Rocha-Lima, and H. Werner,[1] in the study of a single case of verruga in Hamburg, believed that they had found bodies resembling Chlamydozoa in the cells in the skin lesions.

Thus it will be seen from this review regarding the previous investigations pursued, that at the time our studies in relation to this subject were undertaken, the etiology of verruga peruviana and of Oroya fever was undetermined, and the question was in a confused condition, owing to the fact that the work of previous investigators had not been confirmed.

Generally Accepted Opinion Regarding the Nature of Verruga Peruviana

According to the generally accepted opinion among the physicians of Peru at the time of our arrival in Lima, the disease verruga peruviana in the severe form begins with an initial stage known as the *fiebre grave de Carrion* which is characterized by a fever which lasts from fifteen to thirty days, profound anaemia, prostration, and a high mortality. If the patient does not die in this stage the fever begins to abate, and the eruptive, or verruga stage commences. If the eruption is generalized and abundant, then it is stated that the patient is sure to recover. In the chronic or mild form of the disease, which is said to comprise the great proportion of the cases, there is moderate fever of intermittent or remittent character, and pains in the joints are common; more or less anaemia is present. The eruption is said to be the culminat-

[1] Mayer, Rocha-Lima, H. Werner: München. med. Wchnschr., 1913, No. 14.

ing feature in both forms, and it appears under various types which, according to the special characteristics they reveal, are termed " miliary," " nodular," or " mulaire."

Present Investigations

After studying these conditions in Peru, we concluded that verruga peruviana and Oroya fever are two distinct diseases. We have been able to show that the former is due to a virus which may be transmitted to animals by direct inoculation and which produces definite lesions in them, and that the latter is due to an organism parasitic in the red blood-corpuscles and endothelial cells, and sufficiently distinct from the other hematozoa to be placed in a new genus. So far this organism has not been successfully transmitted to the lower animals. The parasite which we consider to be the cause of Oroya fever produces in man fever, and in severe infections a rapid and very pernicious form of anaemia, which results in extreme prostration and frequently in death.

Verruga peruviana, however, is evidently very rarely a fatal disease when uncomplicated with other infections. It is particularly characterized by an eruption upon the skin which may assume a very different appearance in different stages of the disease. Our investigations relating to these two diseases and the evidence upon which these conclusions are based will now be considered in detail.

III

OROYA FEVER

Distribution and Seasonal Prevalence

Our investigations relating to Oroya fever and verruga peruviana were made in Lima, in the large hospitals in that city, and in a number of the mountain towns in the interior of Peru, particularly San Mateo, Chosica, Santa Eulalia, San Bartolomé, Surco, Cocochacra, and Matucana. Cases of verruga peruviana were found in or in the vicinity of all of these towns, and in these localities cases of malaria were also common. Cases of Oroya fever were studied in which the disease had been contracted in or in the vicinity of San Bartolomé and Matucana.

At the present time our knowledge of the geographical distribution of Oroya fever probably is very incomplete. The disease undoubtedly occurs in many of the deeply cleft, narrow valleys situated on the western slope of the Peruvian Andes at altitudes of from 2,500 to 8,000 feet. In these districts, as has been intimated, malaria and verruga peruviana are also very common diseases. Owing to the fact that Oroya fever, or Carrion's disease as it is frequently termed in these localities, evidently has been confused so often with other diseases encountered there, and particularly with verruga peruviana, malaria, and paratyphoid fever, our knowledge of its distribution even in these regions must be far from accurate. Moreover, it would not be surprising if cases of Oroya fever were subsequently found to occur in other tropical countries. Since the parasite of the disease is difficult to detect, and its parasitic nature up to the time of our investigations had not been definitely established, it would not seem strange if the disease has escaped observation in other localities where it exists.

Like malaria, the disease appears undoubtedly to have a seasonal prevalence, and this appears to be more marked than

in the case of verruga peruviana. Oroya fever seems to be much more prevalent in Peru from January to April, and particularly towards the close of the warm, rainy season. According to the hospital reports in Lima, in 1913, more cases were observed, in January, February, and March. In April and May there were no admissions with the diagnosis of this disease at the Dos de Mayo hospital. Our investigations in Peru were largely carried out during the months of June, July, and August, which is the season in which neither Oroya fever nor malaria are particularly prevalent there. However, during these months verruga cases were certainly not uncommon according to our observations.

It is important to emphasize that Odriozola [1] in that portion of his monograph which relates to the *fièvre grave de Carrion,* in discussing the localities in which this disease is encountered asks the question: "Pourquoi, en effet, dans quelques cas, voyons-nous apparaître la fièvre grave de Carrion, tandis que, dans d'autres, c'est l'éruption bénigne qui se présente?" He then goes on to point out just what the climatic conditions and physical features are in these valleys where the severe *fièvre grave de Carrion* is encountered, and concludes a discussion of this part of the subject with the statement: "Par contre, les quebradas qui sont perpendiculaires à la côte jouissent d'une certaine ventilation: elles sont plus ou moins traversées par les vents de la mer et de la Sierra; les variations de température y sont plus fréquentes, à cause de la plus grande facilité qu'a l'air de s'y renouveler. Donc, dans ces quebradas, c'est la maladie de Carrion bénigne, ou de moyenne intensité, qui prendra naissance; et c'est justement ce qui arrive."

These statements from so eminent an authority are of the greatest importance for they seem to suggest to us that the distribution or rather the prevalence of Oroya fever and of verruga peruviana does not always coincide in regard to time and place, any more than does the distribution of verruga peruviana and malaria.

[1] Odriozola: loc. cit., p. 88.

Clinical Features

Clinically the disease is particularly characterized by a fever which may be irregular in its course (see Chart 1, page 21), and in severe infections by a rapid and pernicious form of anaemia which results in extreme prostration and frequently in death. In severe infections the patient may not live longer than three or four weeks from the onset of the symptoms.

Descriptions in the literature regarding the clinical manifestations of this disease are often or, indeed, usually confusing, since almost invariably in these descriptions Oroya fever and verruga peruviana are described as merely different stages of the same disease. The cases of Oroya fever which have terminated fatally are said to have died in the first stage of the disease, verruga, before the eruption has appeared upon the skin. Those cases of true verruga in which an eruption develops are said to be in the eruptive stage of the disease.

Concomitant infections with Oroya fever and verruga peruviana may occur, as we shall presently show, just as concomitant infections with verruga and malaria commonly occur, but the diseases in both instances are entirely independent infections. The occurrence of malarial attacks in individuals during the stage of incubation of verruga peruviana has often led to the wrong diagnosis of the febrile or Oroya fever stage of verruga peruviana, and hence to a confusion in relation to the symptomatology and other clinical features of Oroya fever as described in medical literature. Also it seems evident from the published accounts that cases of infection with paratyphoid fever which have developed verruga peruviana have been confounded with Oroya fever or Carrion's disease. Demonstration of this fact is given by the investigations of several observers. Thus Barton, Biffi, Carbajal, Tamayo, and Gastiaburú have isolated paratyphoid bacilli [1] from the blood of supposed cases of Oroya fever or of Carrion's disease. Obviously it is possible that some cases of Oroya fever are complicated by a paratyphoid infection. It seems

[1] Loc. cit.

evident that it is largely on account of these facts that the published clinical descriptions of uncomplicated cases of Oroya fever are meager.

From the study of the literature relating apparently to uncomplicated cases of this disease, and from our own observations upon other such cases, the following conclusions have been reached in regard to its clinical features.

It is stated that the incubation period is about 20 days, and our observations are not contrary to this idea. In our opinion, however, this statement has not been conclusively demonstrated, and we were unable definitely to verify it, owing to the fact that we did not have an opportunity to observe a sufficiently large number of cases from which to draw definite conclusions. The disease is usually introduced by malaise, pains and weakness in the limbs, and indisposition. Following these symptoms slight rigors and fever appear. The febrile symptoms and rigors usually increase in severity as the disease develops. The fever is often very irregular, being usually remittent, but sometimes intermittent. The temperature frequently fluctuates between 100° F. and 102° F., rarely rising to 104° F. The fever is accompanied by a rapid, pernicious anaemia, which may be complicated or not by hemorrhage, vertigo, or syncope. The number of red cells may be reduced to 1,000,000 per cubic millimeter or less. The skin becomes pale, the mucous membranes assume a waxy appearance, and murmurs are heard over the heart and cervical vessels. Oedema of the legs and about the joints may occur. The tongue is coated, and the bowels frequently constipated in the early stages. Later, diarrhea frequently develops. The urine is scanty, and the specific gravity high. Albumin is occasionally present. The prostration increases as the disease progresses, and restlessness, insomnia, and delirium may develop. In uncomplicated cases there is no eruption upon the skin. It has been assumed by some writers that an eruption in these cases appears in the internal organs. In such an opinion we do not concur, and the results obtained in post mortem examinations do not coincide with this view.

In general it may be stated that the cases of Oroya fever are grave ones and terminate frequently in death. Mild cases which terminate favorably do, however, occur, and we had an opportunity to study infections of this nature. In some instances the temperature sinks to normal or subnormal and death occurs from excessive exhaustion. Death may occur within a few weeks of the onset of symptoms. Some observers report that death may occur as early as ten days from the onset of the attack, but we have not observed instances of such short duration. In the cases which recover, improvement in the severe symptoms of the disease usually begins within twenty-five to thirty days, and convalescence follows. The mortality probably equals usually from 30 to 40 per cent. Some observers however give a mortality of 75 per cent and Rocha-Lima states it may reach 98 per cent. The spleen is frequently enlarged but according to the observations of others is not always palpable. The liver may be moderately swollen, and the lymphatic glands are almost always more or less enlarged. It is stated that pain in the joints is a common symptom, but we were unable to verify this statement in relation to uncomplicated cases of the disease. Pain in the long bones is sometimes complained of.

Blood. The most striking and characteristic feature in Oroya fever is the changes which are found in the blood. This has led to the disease being sometimes termed in Peru "anaemia perniciosa de los quebradas." The development of the anaemia in severe infections may be very rapid and pernicious. In one of the cases studied by us, which will be described presently in detail, the red blood-corpuscles numbered less than 1,000,000 per cubic millimeter, and the entire course of the disease, terminating in death, was but twenty-eight days. Monge [1] reports cases in which the number of red cells fell to 500,000 per cubic millimeter in a very short period of time, and death followed in a few days. Nucleated red cells appear in the peripheral circulation early, and rapidly increase in number. Normoblasts are most numerous, but

[1] Monge: J. Lond. School Trop. Med., 1912, i, Part 3, 239.

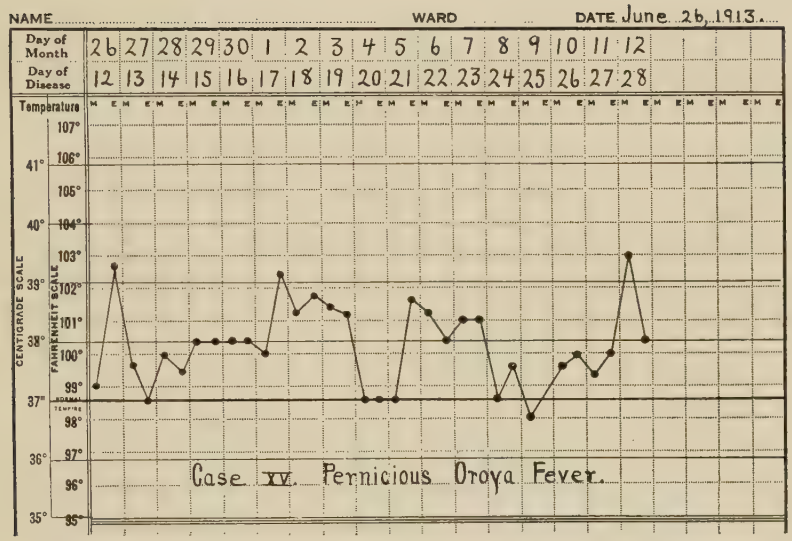
megaloblasts are frequently seen. Polychromatophilia and poikilocytosis are particularly marked in severe infections in both erythrocytes and erythroblasts. Many granular cells are visible. Great variation in the size of the erythrocytes also occurs. Monge who has apparently studied uncomplicated cases of Oroya fever reports in severe ones 2,000 normoblasts and 200 megaloblasts per cubic millimeter. In one case the normoblasts numbered 15,300, and the megaloblasts 3,420. Leukocytosis is almost always present. A count of 20,000 is common. Differential counts often show nothing particularly characteristic in the proportions of the different varieties. Bassett-Smith [1] found in studying the blood from seven cases that the polymorphonuclears numbered from 65 to 70 per cent, the large mononuclears from 7 to 10 per cent, the small mononuclears from 17 to 21 per cent, transitional forms from 1 to 3 per cent, eosinophils from 2 to 4.5 per cent. Biffi [2] found the polymorphonuclears 44 per cent, eosinophils 10 per cent, large mononuclears 16 per cent, lymphocytes 30 per cent. In the severest case which we encountered the polymorphonuclear leukocytes numbered 56 per cent, the large mononuclears 16 per cent, transitional forms 0.1 per cent, small mononuclears 12 per cent, eosinophils 0 per cent: Normoblasts 9 per cent, myelocytes 2.5 per cent. Myelocytes are present in the severe cases ranging usually from 0.5 to 2 per cent. The hemoglobin in severe infections may amount to but 15 per cent. In less severe cases of infection it may be from 40 to 50 per cent. In severe cases the parasites which give rise to the disease are present in the red cells in great numbers, but in mild cases the number of infected corpuscles is small and a long search is often necessary to disclose the parasites. The following notes extracted from cases observed by us will serve to illustrate special clinical features of the disease.

[1] Bassett-Smith: Brit. M. J., Lond., 1909, ii, 783; Lancet, Lond., 1909, 461; Tr. Soc. Trop. Med. and Hyg., Lond., 1914, vii, 158.

[2] Biffi: Bol. Acad. de med. de Lima, 1903, iii, 2.

CLINICAL CHART

NAME ..... WARD ..... DATE June 26, 1913.



Cases Emphasizing Special Clinical Features

Pernicious Oroya Fever

Case 15. Pernicious Oroya Fever terminating in Death. — The patient, aged twenty-three years, a native of Austria, was received in the service of Dr. Arce in the Hospital Dos de Mayo of Lima, on June 26, 1913. We are indebted to Dr. Arce for notes regarding the history of this patient and for the temperature record. The patient came to Peru in January, 1911. In January, 1912, he suffered with malaria and anaemia. In October he had disturbance of the bladder. In April and May of 1913 he traveled on foot through the *quebrada* of Matucana, passing the night at San Bartolomé, which is known to be one of the most dangerous places in relation to disease in the verruga zone. In the middle of June he became sick with chills and fever, which attack he states lasted three days. Eight days later he tried to return to his work but had to abandon it on account of severe fever which was accompanied by pains in the body. The patient took purgatives on several occasions and this was followed by diarrhea and tenesmus. He then grew rapidly weaker. The fever became more intense and headache and delirium developed. He was not able to walk or stand, and was brought to Chorillos on a burro and from there to the hospital on June 26. Upon entrance the patient showed marked anaemia of the skin and mucous membranes. He was emaciated, the skin was dry, the tongue moist and furred. The lungs appeared normal. There was visible pulsation in the region of the heart and the large vessels of the neck. A distinct murmur could be heard in the pulmonary area over the heart. Gallop rhythm was present. Epigastric pulsation was visible. The inguinal and cervical glands were swollen. The axillary glands were just palpable. The liver extended just below the costal margin. The spleen was just palpable and firm to the touch. The patient complained of headache and from the time of entrance suffered with attacks of nausea, vomiting and diarrhea. The faeces were fluid and contained bile. Ova of *Trichocephalus trichiurus* were present in the faeces. The analysis of the urine showed a small amount of albumin and urobilin present. The patient gradually sank, a systolic murmur appeared over the apex of the heart and diarrhea increased. A moderate oedema of the legs and hands appeared.

On July 7 the following note was dictated by one of us:

" Patient in grave condition, temperature 100°.7 F. The skin presents most extreme grade of pallor. There is very marked anaemia of the mucous membranes and finger tips. Hemic murmurs are heard at the apex of the heart and over the cervical vessels. About 10 c.c. of blood were drawn from the vein of the forearm and cultures were made on various

media. Microscopical preparations were also made of blood from the ear. Many of these were fixed and a few were examined as fresh preparations. Motile parasites were observed in the red cells in the fresh preparations and also in stained ones. The Wassermann reaction is negative to beef heart antigen. The red blood-corpuscles measure approximately 500,000 to the cubic millimeter. It is difficult to count the red blood-corpuscles on account of the fact that they become massed together in rouleaux. The white blood count is 27,000 cells to the cubic millimeter, without correction for the normoblasts; the hemoglobin 15 per cent (Sahli); the urine shows a small amount of albumin, and the faeces, ova of *Trichocephalus trichiurus*."

On July 8 the following note was made:

"Specimens of fresh blood again examined from 8 to 11.30 A.M. Definite organisms probably allied to Anaplasma were observed within the red cells. These were very numerous, and consisted of rounded bodies and also of elongated rod-like or somewhat spirillum-like bodies. The rounded bodies though not possessing Brownian movement, nevertheless moved about in the red cells, and frequently changed their relative position to one another. In some cells both rods and rounded bodies could be observed. The number of rounded bodies in the cells frequently equaled three or four. The rod-shaped bodies were also frequently multiple. They possessed distinct oscillatory and undulating movements, rotating, and freely changing their position within the blood-corpuscle. The rod-like bodies were not only straight, but some were curved and some were almost spirillum shaped. In one corpuscle which was watched for one hour and fifteen minutes there were two rounded bodies and one rod-shaped body. The rod-shaped one possessed distinct oscillatory movement, changing its position freely in the protoplasm of the cell, while the two rounded bodies also changed their position within the body of the cell, but to a much less extent. At times the rod-like body had distinct undulatory movements, and appeared to be making attempts to escape from the periphery of the red cell as it moved from the center to the border of the red cell. During one period of the observation one pole showed a distinct drumstick-like appearance. At one time the head of the drumstick seemed to touch one of the rounded bodies, and then a very sudden vibratory motion occurred throughout the rod-shaped body, the extremity lashing back and forth. It then became again free. About half an hour later this rod-shaped body seemed to have become much shorter, reaching then only about half its former length, and at both poles there appeared to be a slightly swollen end. The rod-shaped body gradually became quieter, there was no more oscillation, and it became increasingly difficult to see. At times it appeared as though it was more in the depth of the corpuscle

than at others. It was watched for a half-hour further, no other change occurred in the cell, except that the bodies became very difficult to distinguish at all. The rounded bodies seemed to consist of two crescentic, more opaque bodies almost touching one another, surrounded by a more translucent, rounded, grayish area."

On July 9 the following observation was made:

"The fresh blood shows apparently a smaller number of the rod-shaped and rounded bodies. The rounded bodies seem to consist of a vacuole-like space in which is contained a small, dot-sized, less translucent area. In stained preparations these appear as a small, dot-sized, compact mass of chromatin contained in a vacuole-like space evidently representing a protokaryon of the smallest type. The study of the bacillary bodies today shows that when the slide is warmed before the blood specimen is taken, and the preparation examined immediately, the bacillary forms have an oscillating movement within the red corpuscle. They appear to be made up of soft protoplasmic structure, and when they move, the limiting longitudinal edges of the organism do not remain as straight lines, and the organism appears at these times to have a greater width in some places than in others. The bacillary forms in the fresh preparations frequently appear bead-like at both ends. The movement of the bacillary bodies is entirely unlike that of pedesis, but is an oscillation of the protoplasm itself. The organism sometimes revolves around the entire circumference of the red cell. In some of the preparations, which were taken upon a cold slide, the movement of the organisms was either not visible or became suspended after one or two minutes."

Numerous parasites were also found in the stained blood specimens, a detailed description of which will be found on p. 29 of this report. On July 12 the patient's temperature rose to 102°.7 F. The diarrhea and vomiting had persisted. The tongue was dry and furred. The patient grew weaker and weaker, the pulse became rapid and finally could no longer be felt, and he died at 2 P.M. The description of the necropsy is given on page 46.

Concomitant Infection

Cases of verruga peruviana, Oroya fever, and malaria are frequently found in the same endemic regions, and we have referred to the fact that many cases of verruga peruviana are complicated by malaria. The statistics of Peruvian physicians demonstrate conclusively this fact, as do our own observations upon cases of verruga peruviana. Sometimes concomitant

infection with verruga peruviana and Oroya fever occurs. The following case observed by us is one of this nature:

Case 20. Mild Case of Oroya Fever complicated with Verruga Peruviana. Recovery. — Patient was first seen in San Bartolomé on July 4. At this time he showed a marked anaemia and complained of fever. The mucous membranes showed marked pallor, and the skin was distinctly pale. The physical examination of the chest showed nothing abnormal and there was no visible eruption upon the skin. There were many cases of verruga peruviana in San Bartolomé and in the vicinity of his dwelling at the time. The red blood-corpuscles numbered 3,300,000; the white corpuscles 4,500; the hemoglobin 50 per cent (Sahli); the specific gravity of the urine was 1.023, but nothing abnormal was observed in it. A blood smear was made from the case, and we were compelled to return by train a few minutes later to Lima. The preparation of the blood was examined in Lima, and found to contain a few parasites (*Bartonella bacilliformis*). On July 23 the patient was seen again for a few minutes and blood smears were again taken. The patient still showed marked anaemia, pallor of the skin and of the mucous membranes. He stated that he was growing weaker, though still sitting up most of the time. On the legs above the ankles four or five reddish papules measuring from 2 to 4 mm. were present. One was of a dark cherry color, the skin over it appearing tense and translucent. There was also one lesion measuring 3 mm. in diameter on the right forearm. These evidently represent lesions of verruga peruviana. On returning to Lima *Bartonella bacilliformis* was found in small numbers in the stained preparations of the blood. The patient refused to come to the hospital or to accept medical treatment, and would only allow a few preparations of his blood to be taken from the ear. On August 1 we again visited San Bartolomé and saw the patient and collected microscopical preparations of the blood. No parasites were found in these blood preparations.

The following note was made:

"The eruption of verrugas has become considerably more profuse and distinct. The papules are now situated over both surfaces of the right and left forearms. They measure from 2 to 6 mm. in diameter, are red in color, and raised above the surface of the skin. They are quite typical of verruga, and there are about thirty of the larger ones on both forearms. The palms of the hands and soles of the feet are clear. There are also about twenty papules on the forehead and left side of the cheek, measuring 2 to 3 mm. in diameter. On the legs there is a similar papular eruption, particularly on the anterior surface over the lower portion of the tibia. One of the lesions measures 1 cm. in diameter. The others are small in size. The patient still looks pale and he complains of much pain in his

legs." He again refused to enter the hospital or to allow any of the lesions of the skin to be removed for examination. It was not possible to see this patient again, but we understood that he eventually recovered. At the time he suffered from Oroya fever there were in his immediate neighborhood eight cases of verruga peruviana.

Mild Infection with Spurious Eruption

In Peru in the regions where Oroya fever and verruga peruviana prevail the inhabitants are all very familiar with the disease known as verruga, and with the inoculation experiment of Carrion. It is the popular and wide-spread belief in Peru that Carrion inoculated himself with blood from a case of verruga, and died of Oroya fever, subsequently often known as " Carrion's disease." The inhabitants in these regions stand in much fear of the febrile stage of Carrion's disease, and there is a general and implicit belief among them that if the eruptive stage of the disease can be brought on by treatment, that the individual will then surely recover. For this reason when cases of severe fever and anaemia occur various remedies are employed by the natives with the idea of causing this eruption to develop. In this way, by the use of rubefacients and other substances irritating to the skin, very striking cutaneous lesions are sometimes produced. If the individual recovers from the attack of fever, then it is claimed that the recovery from the fever has occurred because a copious and abundant eruption has been produced upon the skin. Obviously many fevers of different origin are treated in this way. Moreover every papule or mole or naevus upon the skin overlooked in the previous incomplete examination, but subsequently detected, is pointed out enthusiastically, frequently even by nurses and doctors, as an evidence of the appearance of the eruption. Nothing further for the diagnosis of verruga seems necessary in such cases.

The following case illustrates these facts:

Case 8. Moderately Severe Oroya Fever terminating in Recovery. — The patient, an Italian twenty-seven years of age, was first seen on July 4, 1913, in San Bartolomé, Peru, a well known endemic center of Oroya fever, verruga peruviana, and malaria. He gave a history of having been in

San Bartolomé for seven weeks and having had fever for three weeks. He was employed as a workman upon the railway until he became sick. Physical examination of the chest revealed nothing abnormal. The spleen was not definitely palpable and the liver did not extend below the costal margin. The temperature at the time was 101°.3 F., the pulse one hundred. The mucous membranes were pale in color. The patient was distinctly anaemic. A thorough examination of the skin showed one small papule measuring 2 mm. in diameter on left wrist, grayish in color, very slightly raised, and rather dry. No signs of an inflammatory process in it were visible and it apparently had little significance. On the skin over the anterior surface of the tibia there was a small papule measuring about 3 mm. in diameter, and slightly raised. It was gray in color and appeared as an old dry naevus. No other lesions were visible on the skin. Fresh and stained microscopical preparations were made from the blood and 20 c.c. were withdrawn from the median basilic vein and animals inoculated at once. (These inoculations will be referred to elsewhere in the report. See page 36.) The red blood-corpuscles numbered 3,100,000; the white blood-corpuscles, 13,000; the hemoglobin 40 per cent (Sahli).

The differential count in this patient was not as characteristic of Oroya fever as Case 15, partly because the anaemia did not progress to an extreme grade and also because the blood picture was complicated by extensive application to the skin of turpentine and other rubefacients. This treatment was probably responsible for the appearance of moderate amounts of albumin in the urine and it might easily have modified the blood picture. The differential count is as follows:

Polymorphonuclear neutrophils	72 %
Large mononuclears	10
Lymphocytes	18
Normoblasts	0.3
Myelocytes	none

The examination of both fresh and stained preparations of the blood showed a few parasites of Oroya fever (*Bartonella bacilliformis*). The Wassermann reaction was negative. The patient refused to be taken to the hospital in Lima or to accept medical treatment, saying he preferred to be treated by an old native woman who had had experience in treating verruga cases. It was therefore necessary to leave him in San Bartolomé and to return to Lima. The patient was seen again on July 23 at San Bartolomé. During the interval he was nursed by his brother who, under the direction of the old woman referred to above, carried out the instructions prescribed by her.

The following note was made on this date:

" His brother reports that he has been much improved during the past week, and has had less fever. He has been rubbing him with leaves of

Fig. 1

Parasite of Oroya Fever in fresh blood specimen, showing changes in shape and position in the cell that the organism underwent during a period of 1½ hours in which it was observed.

Fig. 2

Parasite of Oroya Fever
in fresh blood specimen
(bacillary and rounded forms).

PLATE IV — OROYA FEVER

several kinds, some of which were examined in a fresh state, and found to be those of the castor oil plant (*Ricinus communis*). Examination of the patient shows vesicular dermatitis over the face and legs, where the leaves have been rubbed. The leaves rubbed on evidently have had powerful properties, and have produced lesions resembling strongly those due to *Rhus venenata*. His brother states that these are verrugas which he has caused to come out by the treatment. There is no resemblance, to an experienced eye, in these lesions to those of verruga peruviana. Blood smears and fresh preparations were made from the patient's blood, which were examined in San Bartolomé, and also at the next station a half-hour later. In the fresh preparation only two parasites were found, one of which was observed for an hour and a half. This observation was made at Surco, where no chair or table was obtainable, and it was necessary to place the microscope on a barrel, and stand while the preparation was studied. In the field of the microscope, which was watched for an hour and a half, only one infected corpuscle was visible. The other corpuscles in the field appeared normal, though there was some variation in size. There was but one polymorphonuclear leukocyte in the field which was not pigmented. The parasite was situated in a cell which appeared normal in other respects. It was bacillary in form, and had an undulating movement similar to that observed in the rod parasites in the other cases of Oroya fever. At times it was difficult to see the parasite, as it seemed to be deeper in the corpuscle. At other times it was quite sharp. On focusing, the center of the rod seemed more dense and opaque (darker), and as though lying in a vacuolated space. At the poles sometimes it appeared as though a dot or bead were visible, but this was not distinct. The parasite moved about the cell slowly, and, as may be seen from the diagrams which were made in order (Plate IV, Fig. 1), it in turn occupied almost every part of the cell. At one time it was observed just at the periphery; at another, it was perfectly straight; again it was curved, or rather sharply bent in the middle; at other times bent as two commas joined together, never markedly S-shaped, and never pear-shaped, but occasionally slightly thicker in different portions of the body when moving. The movements were slow, and never suggested Brownian movement in any way. The undulations gradually became slower, but otherwise the picture did not change during the hour and a half of observation, when it became necessary to take up other work."

On August 1 the patient was again seen. The following note was then made:

" The face, hands, forearms, and feet and legs are swollen and more or less puffy. There is large amount of dry serum on the surface of the skin, and numerous scabs which have formed owing to the breaking of follicles produced by the rubefacients used in treatment. Turpentine, leaves of

the castor oil plant, and other leaves have been used to rub the skin. The patient has also been given various concoctions to drink. On the legs there are also numerous small pin-point and pin-head sized hemorrhages, which are probably the result of irritation and scratching. The mouth and lips are greatly swollen and covered with sordes. The mouth has an appearance as though the patient had been salivated. The tongue is swollen, and there are several small fissures at the edges. On the mucous membranes of the gums and the palate there are numerous, closely placed, small (about 2 mm. in diameter) nodular areas. On the glans penis there is a mass measuring about 1 cm. in diameter, composed of similar small nodules which are all confluent. It appears not unlike a soft chancre. The patient says he has no fever, but refuses to allow any examination to be made. Owing to the treatment he has received it is impossible to form a definite conclusion regarding the lesions in the mouth and on the penis. All the lesions present on the skin could be explained as the result of the treatment." We were also denied admittance to this patient subsequently but were informed that he also recovered.

Description and Classification of the Parasite of Oroya Fever (*Bartonella bacilliformis*)

A review of the various opinions regarding the bodies observed in the red blood-cells in Oroya fever has been given on pages 10–13.

The parasites giving rise to the disease were observed by us in the peripheral blood of Oroya fever cases both in fresh and in stained preparations. The organisms consisted of minute rods and more rarely of rounded bodies, situated within the red cells. Subsequently other stages of development were discovered in the endothelial cells of blood-vessels, particularly those of the lymphatic glands, liver, and spleen.

Fresh Blood Preparations.— Although in fresh blood preparations the organisms are frequently difficult to detect, and at times lie deep in the substance of the red blood-cell, nevertheless with good illumination and an oil-immersion apochromatic objective, at times they may be distinctly observed. In form they are rounded or rod-shaped, though the rods are not always straight in outline. The rods measure approximately from 1.5 to 2.5 μ in length and the rounded bodies from 0.5 to 1 μ in diameter. In severe infections, red corpuscles in almost

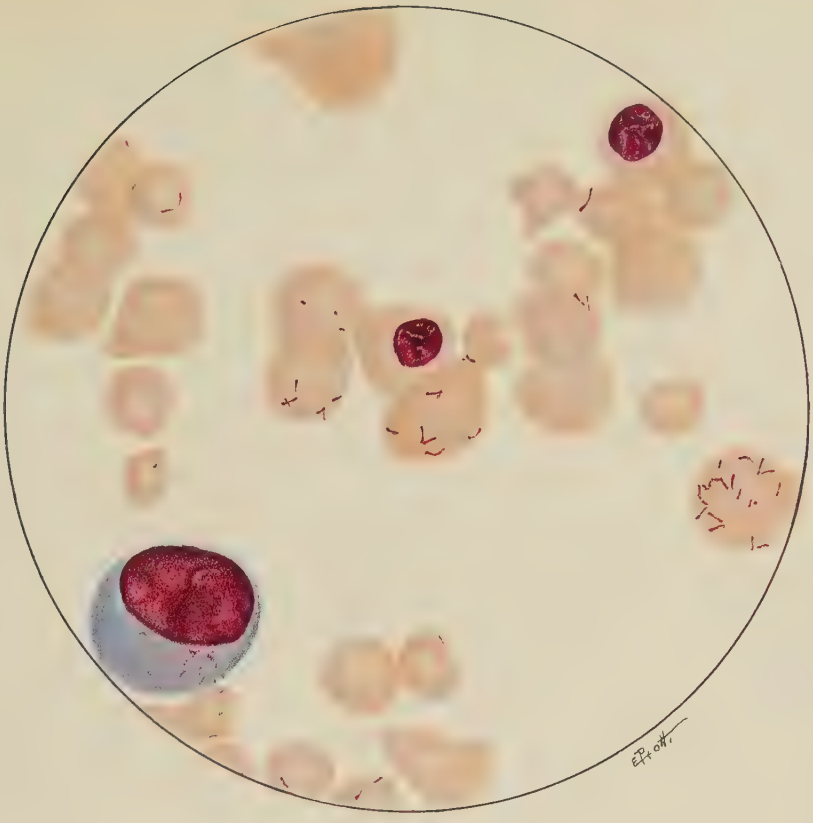
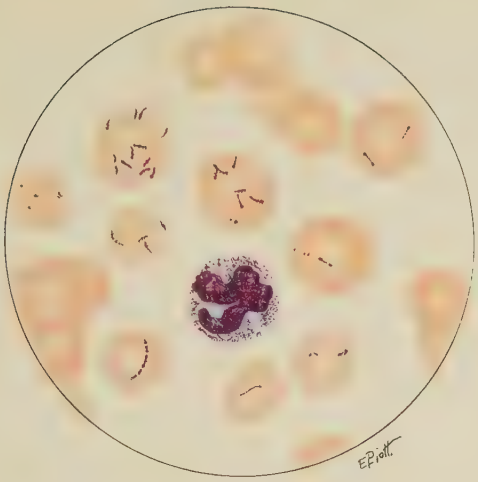


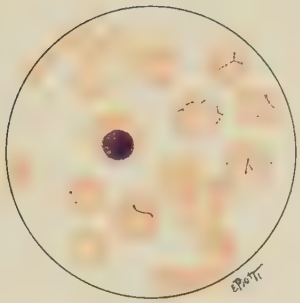
Fig. 1

Fig. 2

PLATE V — PARASITE OF OROYA FEVER

in stained blood specimens.





Figs. 1 and 2

Parasite of Oroya Fever
in stained blood specimen.

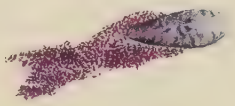


Fig. 3

Isolated endothelial cell.

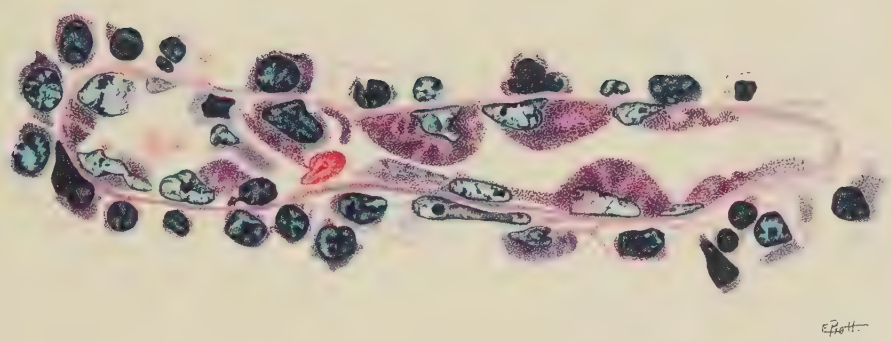


Fig. 4

Section showing cycle of Bartonella bacilliformis
in endothelial cells of lymphatic gland.

PLATE VI — OROYA FEVER

every field of the microscope are invaded by the parasites, and numbers of both rounded bodies and bacillary forms are frequently observed in a single cell. The organisms are endowed with definite motility, which amounts to slow transition and is totally distinct from that of pedesis. To observe this it appears advisable previously to warm the slide and to examine the preparation immediately after it is made. In red cells in which several of the parasites are visible it is easy to observe their frequent change of position within the cell and with reference to one another. The rod-shaped forms were observed to glide slowly in the direction of their long diameter and to exhibit a slight bending in their transition in the red cell, and at times both these and the rounded bodies might be seen to occupy in turn all portions of it. They were never observed to appear as distinct cross forms, or as spirals or markedly S-shaped. Occasionally at the two poles of the organism a dot or bead-like appearance was observed. On account of their small size the rounded bodies are more difficult to describe in fresh specimens and it can only be stated that they change their position within the cell. A more detailed account of the movements of the parasite is given in the notes on pages 22, 23, and 27. See also Plate IV.

Stained and Fixed Preparations. — The rod-shaped forms measure approximately from 1 to 2 μ in length and from 0.2 to 0.5 μ in thickness. They are frequently slightly curved, and occur singly or end to end in pairs, or in chains of three, four and five. When numerous they often lie parallel to one another. V forms, probably representing dividing organisms, are frequent. Y forms are also not uncommon. Cross forms are rare and may be due to organisms being superimposed. The ends of the rods in stained preparations are often more intensely colored. Single rods sometimes show a deep red or purplish granule which may be of the nature of chromatin and which gives the appearance of a swelling at one extremity, the rest of the rod having a more bluish tint, sometimes slightly deeper at the end opposite the one showing the granule. Other rods may be blue throughout or have a deeply stained

granule at each end. In organisms occurring in chains the deeply staining granules sometimes give a beaded appearance to the chain (Plate v). It is evident that the organisms do not all lie in the same plane within the corpuscle, and that many of them are not superimposed upon it, as has been reported by Lawson [1] to be the case with the malarial parasite. This is evident from some of the photomicrographs.

The rounded forms measure roughly from 0.3 to 1 μ in diameter. While many of these are rounded, others are slightly oval, or suggest pear shapes. They occur singly, in pairs, or in groups which suggest previous division. (Plates v and vi.)

The red cell may contain a variable number of parasites. In severe infections from one to ten is not unusual, as may be seen from the photomicrographs (Plate vii). Nucleated red cells, at various stages of development, are of frequent occurrence, and are sometimes infected with the parasites (Plate vii, Fig. 4). From the anaemia which occurs in this disease and the number of red cells infected with parasites, it seems evident that the red cells containing the parasites are ultimately destroyed.

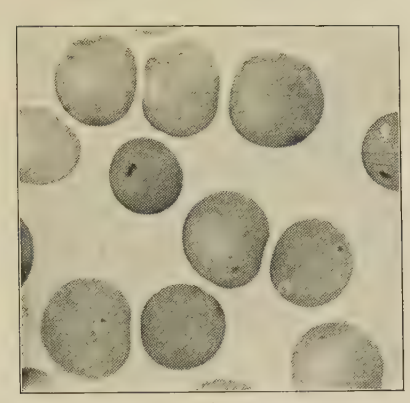
Classification

From the description given above of the parasite as observed in the red blood-corpuscles it appeared that we had to do with a species of organism possessing some of the characteristics described for Anaplasma or of Theileria, but also differing in other characteristics described for each of these genera. The rounded bodies, in their form and size, and in the fact that they apparently consist entirely or almost entirely of chromatinic substance, resemble certain bodies which were first observed by Theobald Smith [2] and later by Theiler [3] in the red blood-corpuscles of cattle afflicted with Texas fever. Similar forms were subsequently found in the red blood-corpuscles of other animals afflicted with disease, and these bodies are now classified as Anaplasmata. The bacilliform

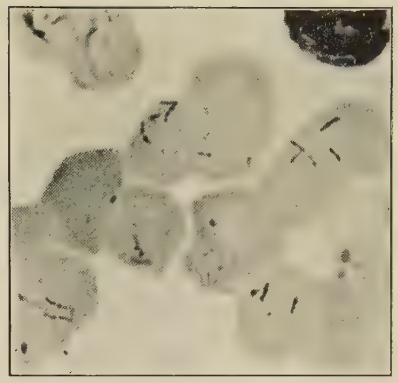
[1] M. Rowley-Lawson: J. Exper. Med., 1913, xvii, 324.

[2] Smith: Rep. Bureau Animal Indust., Wash., 1891–92, 177, and Plate iv, Figs. 1, 2, 3, p. 302.

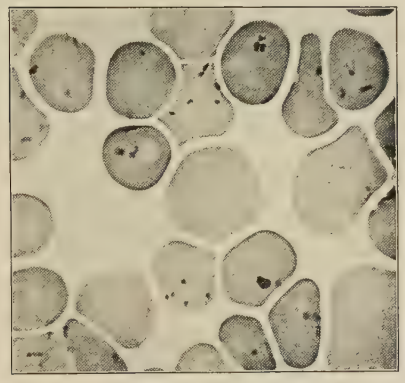
[3] Theiler: Ztschr. f. Infectionskrankh. der Haustiere, 1910, viii, 39.



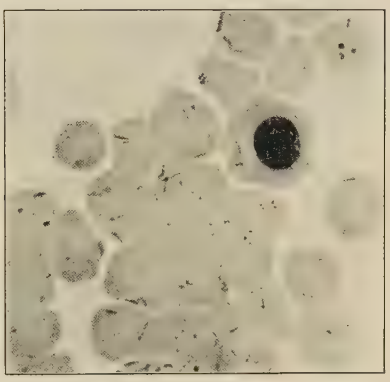
1



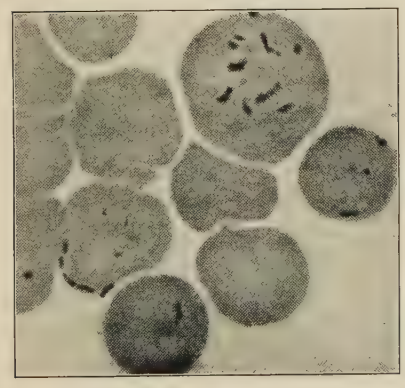
2



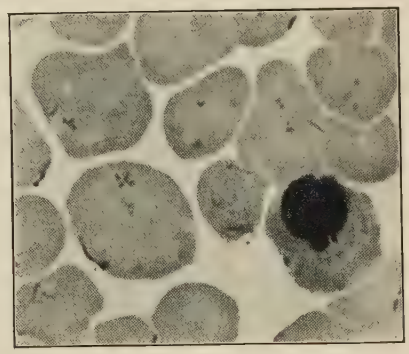
3



4



5



6

FIG. 1. — Mild infection. FIGS. 2, 3, 4, 5, 6. — Severe infection.
FIGS. 1, 2, 3, 4, 6. = × 1500. FIG. 5. = × 2000.

PLATE VII. — OROYA FEVER PARASITE IN RED BLOOD-CELLS (STAINED PREPARATIONS).

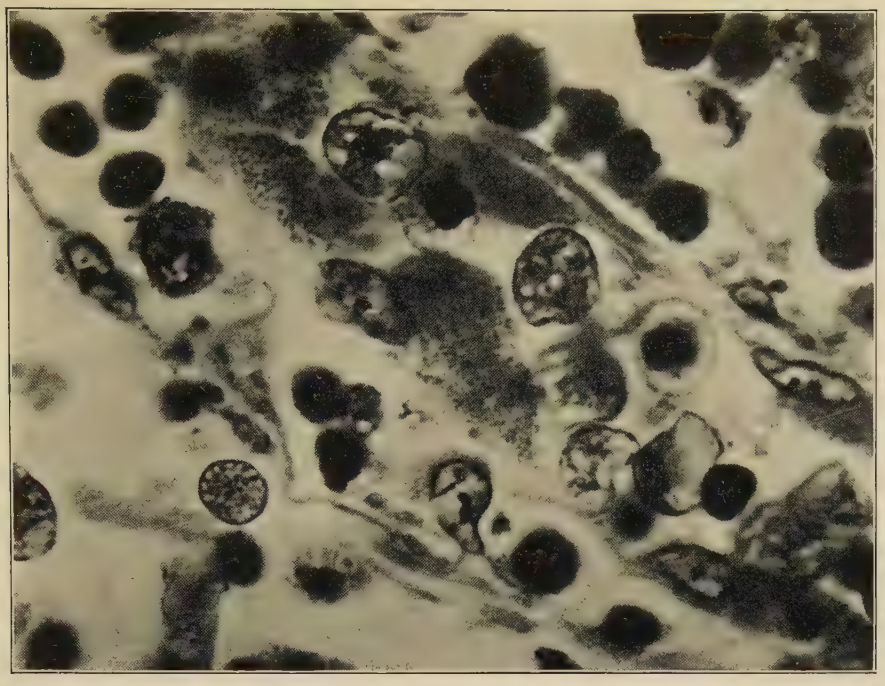


FIG. 1. — LYMPH-NODE. Showing swollen endothelial cells in blood-vessel wall.

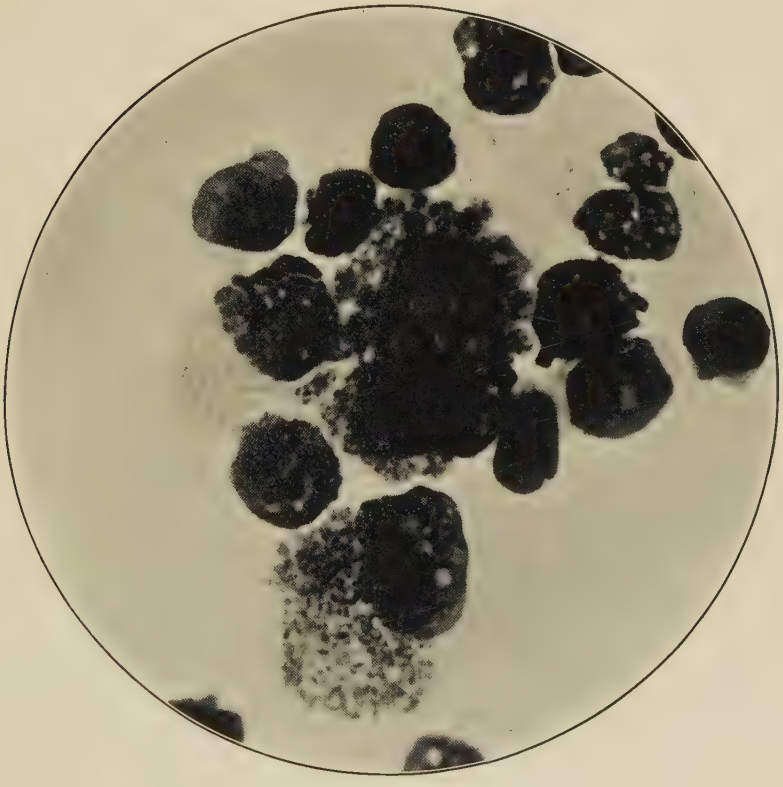


FIG. 2. — SMEAR FROM LYMPH-NODE. ENDOTHELIAL CELLS.
Showing phase in multiplication of parasite. Smear stained by Giemsa's method.

PLATE VIII. — OROYA FEVER.

or rod-shaped bodies predominate in our specimens, and in their morphological appearance some of them resemble considerably the rod-shaped bodies observed in certain species of Theileria. However, in their staining reactions they are somewhat different, the chromatinic substance is not differentiated from the cytoplasmic substance with the same ease, and distinct cross forms have not been observed. Moreover, their movements, so far as could be observed, were unlike those described for the schizonts in the Piroplasmata. While the organism at first sight might be regarded from its morphology alone as a species of bacterium, this idea is not supported by further study. It is essentially a parasite of the red blood-cell; attempts to cultivate it on various culture media have been unsuccessful and the inoculation of large amounts of blood from severe cases of Oroya fever has failed to infect a monkey or rabbits.

From the evidence available at the time of our first publication on the subject it appeared that the organism observed in the blood in Oroya fever might belong to a group of microörganisms intermediate between the protozoa and the bacteria, just as perhaps the Spirochaetae form another such group. It resembled in some of its characteristics the features given for two species of Grahamella N. G. Protista described and classified by Brumpt [1] in October, 1911, although in preparations stained by Giemsa's or Wright's stains the presence of reddish-stained granules and of bluish cytoplasm in many forms favored its relationship to the protozoa. We regarded it preferable, at that time however, until further information as to its nature was acquired, to follow Brumpt in his classification of Grahamella, and to go no further than merely to place this species for the time with the Protista. From the descriptions in the literature it appeared that the bodies described by Graham-Smith,[2] Prowazek,[3] and others in the

[1] Brumpt: Bull. Soc. d. pathol. exotique, Par., 1911, iv, 514.

[2] Graham-Smith: J. Hyg., Camb., 1905, v, 453; Thomson: ibid., 1906, vi, 574; Balfour: Report Wellcome Trop. Research Lab., 1906, ii, 111; 1911, iv, 120; Bull. Soc. d. pathol. exotique, Par., 1911, iv, 660; Leger: ibid., 1913, vi, 247.

[3] Prowazek: Centralbl. f. Bakteriol. u. Parasitenk., Jena, 1913, lxx, Orig., 34.

blood of moles and other animals and classified by Brumpt [1] and Prowazek [2] as parasites, might constitute organisms of species related to the one which we had encountered in the blood in Oroya fever. As in the genus Grahamella the organism of Oroya fever is characteristically rod-shaped, and evidently multiplies in one stage by binary transverse division. However, the resulting elements are sometimes observed in chains. On the basis of this difference and of other additional characteristics in the life cycle, motility, staining reactions and size, it appeared preferable to propose for this organism the name of *Bartonia bacilliformis*. The generic name of Bartonia was suggested owing to the fact that Barton in 1909 inclined to the belief that the inclusions earlier observed in the red cells were protozoa.

Dr. P. W. Bassett-Smith has since kindly called the attention of one of us to the fact that the name Bartonia had been used as a generic one in botany to include two species of Gentianaceæ. This use would not necessarily preclude its employment in zoölogy although a duplication of names in botany and zoölogy is certainly highly undesirable. Upon investigation, however, we have found that the use of Bartonia as a generic name has been applied by Cossmann [3] to an eocene mollusk, *Bartonia canaliculatum*, hence we have given the generic name "Bartonella," retaining the specific one, "bacilliformis" (*Bartonella bacilliformis*).

The genus was also defined provisionally from the forms studied in the red blood-corpuscles as follows: organisms rounded or rod-shaped, sometimes occurring in chains of several dividing forms; reproduction by binary transverse division; cytoplasm and chromatinic substance often differentiated with difficulty; endowed with independent motility; living as parasites in the red blood-corpuscles.

Development within the Endothelial Cells.— Subsequently upon examining sections of the organs from severe fatal cases of Oroya fever, in addition to the pathological changes ob-

[1] Brumpt: loc. cit. [2] Prowazek: loc. cit.

[3] Cossmann: Essais de Paléoconchologie Comparée, 4me Livr., Paris, 1901.

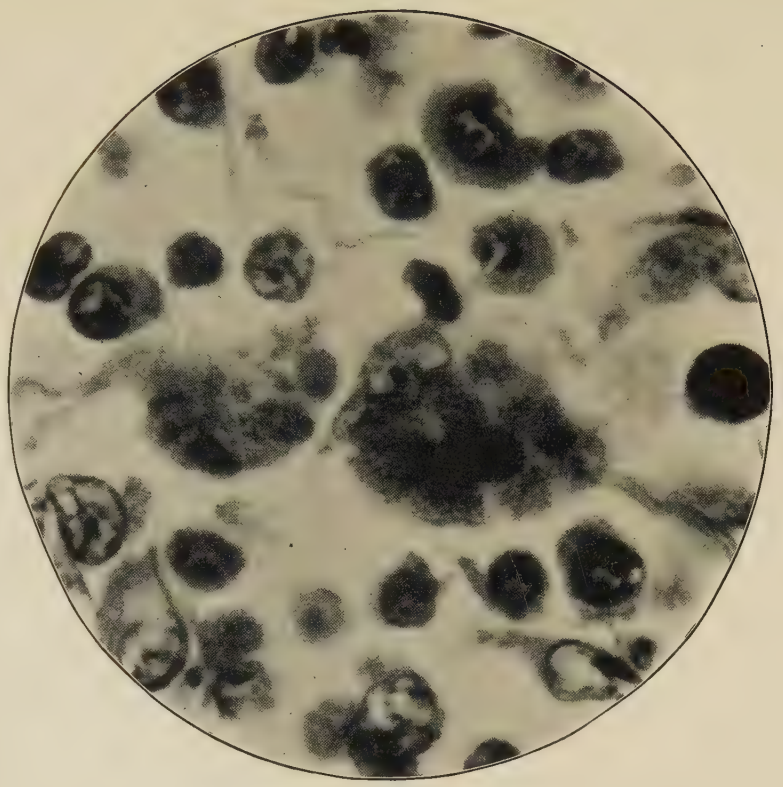


Fig. 1. — Endothelial Cell in blood-vessel of lymph-node.
Showing parasite in form of rounded bodies with minute chromatin granules.

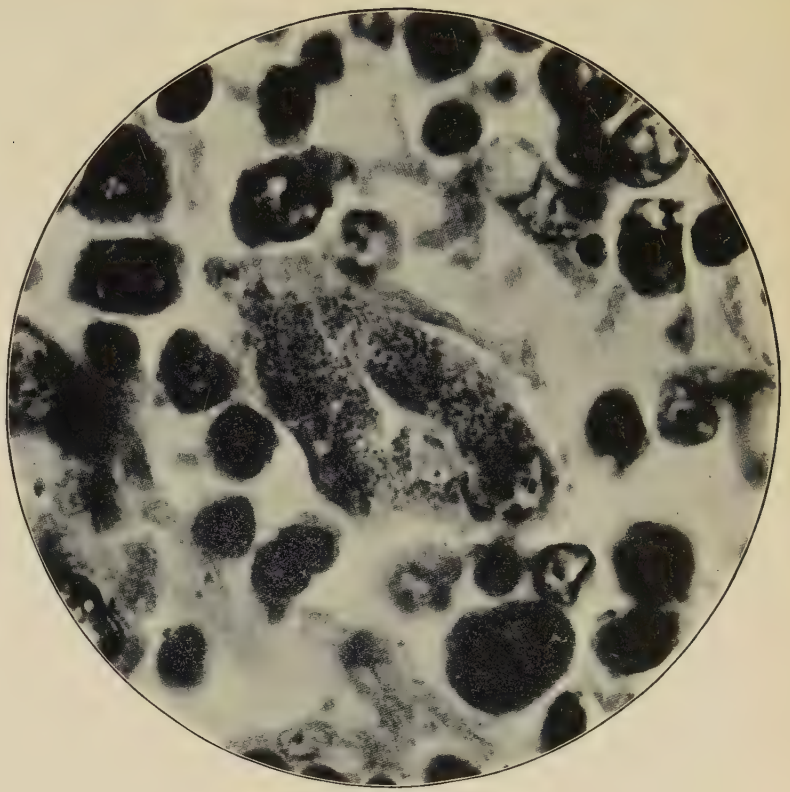


Fig. 2. — Endothelial Cell in blood-vessel of lymph-node.
Showing later stage of parasite, i.e., rod forms.

Plate IX. — Oroya fever, Lymph-node.

served in the other organs and described on page 49 of this report, other evidences of the life cycle of the parasite were observed in the swollen endothelial cells of the lymphatic glands and spleen. In the lymphatic glands the most striking histological feature is the presence of large, swollen, endothelial cells, sometimes free in the lumen of the blood-vessel, at other times still attached to the wall. (Plate VIII.) In some instances a single endothelial cell is so distended that it will almost occlude the entire lumen of the vessel. These cells are distended with large numbers of rounded and rod-shaped bodies and many of them are in various stages of degeneration. Upon the study of smear preparations made from the lymphatic glands and stained by Giemsa's method, the following observations were made. The endothelial cells in these preparations contain rounded bodies with bluish cytoplasm and small granules of chromatin (Plate X). In some of these bodies but a single granule is present; in others, two, four, or many may be observed. Sometimes the cell is distended with rounded masses or spheres each of which contains a definite number of minute points of chromatin. (Plate X, Figs. 2 and 3.) These spheres are of quite uniform size. From a study of other cells it would appear that these spheres break up into a large number of minute elements each of which contains a chromatin granule; these become elongated and finally appear as distinct rods containing at one end a minute particle of chromatin. (Plate X, Fig. 5.) This latter appearance is particularly apparent when the sphere is ruptured and the rods set free. If these rod-shaped elements, which have been set free by the rupture of the endothelial cells, are compared with the rods found within red blood-corpuscles lying near, it is seen that they are identical in character. Moreover they are entirely similar to the forms already observed in the blood-cells in the peripheral circulation. It seems very probable that the rods which have escaped from the spheres constitute gametocytes, rather than merozoites, which have subsequently entered the erythrocytes, and the movements of the parasites observed in

the red blood-corpuscles in fresh preparations, and already referred to in this report, coincide with this idea. Thus the appearances observed in the endothelial cells of the lymphatic glands from Oroya fever cases resemble considerably those described and pictured by Gonder [1] in his description of the life cycle of *Lympho-haematocytozoön parvum* (formerly *Pirosoma parvum*), (Plate xi, Fig. 1). Through the kindness of Dr. K. F. Meyer one of us recently received microscopical preparations of this organism, and from a study of these preparations and the articles by Meyer [2] and Gonder upon this subject, the similarity between the organism observed by us in the endothelial cells in Oroya fever and *Lympho-haematocytozoön parvum* is further suggested. Owing to the more minute size of *Bartonella bacilliformis*, however, particularly in the multiplicative phase, its life cycle within the endothelial cells is much more difficult to observe and interpret. From these observations, it appears that *Bartonella bacilliformis* should be classified as a protozoan and that it is related to the Lympho-haematocytozoa, a genus recently created by Meyer. However further observations upon a larger amount of material are desirable.

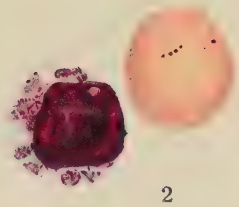
Attempts to Infect Animals with the Parasite of Oroya Fever (*Bartonella bacilliformis*)

Numerous attempts were made to infect animals with the parasite observed in the blood of Oroya fever cases. These experiments are of particular interest and importance in relation to the differentiation of Oroya fever from verruga peruviana, and form one link in the chain of evidence that the two diseases are distinct and have an entirely different etiology. Our experiments show that we were able with little difficulty to infect animals with the virus of verruga peruviana, but our attempts to inoculate animals with *Bartonella bacilliformis* were unsuccessful. The experiments undertaken to inoculate animals with this haematozoön will first be outlined.

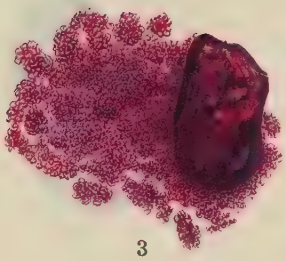
[1] Gonder: J. Comp. Path. & Therap., Edinb. & Lond., 1910, xxiii, 328.

[2] Meyer: Kolle & Wassermann, Handb. d. Path. Mikroorganismen, Jena, 2. Auflage, 1913, vii, 531, 539.

1

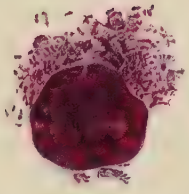


2

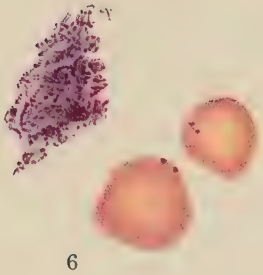


3

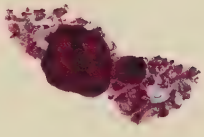
4



5



6



7

PLATE X — OROYA FEVER

Endothelial Cells

showing various stages in the development of the parasite;
early rounded forms later breaking up into rods.

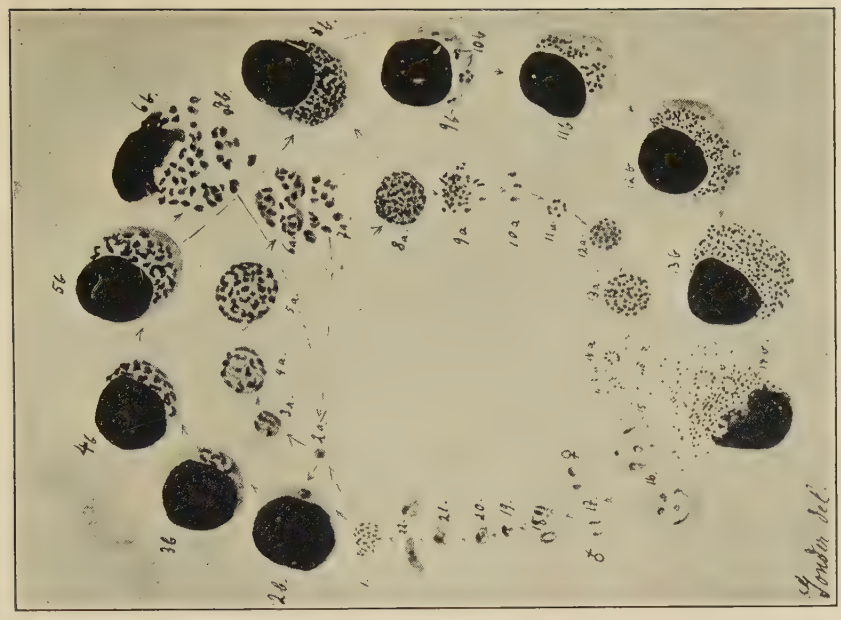


Fig. 1. — Life cycle of Lympho-haematocytozoön parvum.

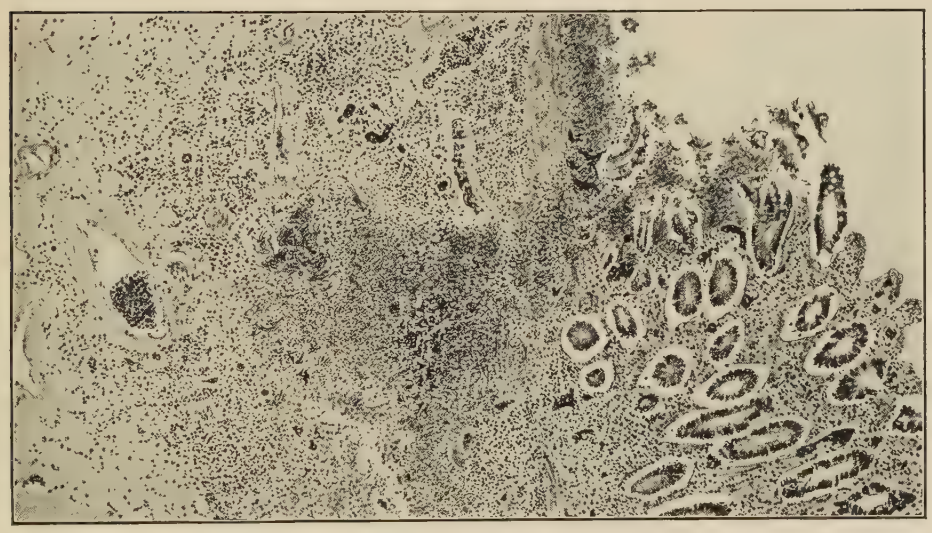


Fig. 2. — Ulceration in large intestine.

Plate XI. — Oroya Fever.

Testicular inoculations in rabbits were first made with the idea of detecting or of excluding spirochaetal infection. Testicular inoculations with the virus of verruga peruviana produce definite lesions but not of spirochaetal origin.

Experiments

I. July 7, 1913. 10 c.c. of blood were drawn from the median basilic vein of a severe case of Oroya fever (Case 15) which resulted fatally five days later. The microscopical examination of the blood on this date revealed a very severe infection with *Bartonella bacilliformis*. Animals were inoculated with the blood at this time and the notes of the experiments are as follows:

Rabbit No. 33. — Testes inoculation with Oroya blood.

July 7, 1913. Both testes (small) inoculated with 0.5 c.c. of defibrinated blood of case at Dos de Mayo Hospital about 1½ hours after removal.

July	9.	11.45 A.M.	Temp.	101°.6 F.	Testes	small,	normal.		
"	11.	12.15 "	"	102°.3.	"	"	firm,	normal	
"	15.	11.35 "			"	"	"	"	
"	18.	12 M.	"	102°.	"	"	"	"	
"	21.	11.40 A.M.	"	102°.5.	"	"	"	"	
Aug.	3.				"	"	"	"	

Rabbit No. 34. — Testes inoculation with Oroya blood.

July 7, 1913. Both testes each inoculated with 0.5 c.c. of defibrinated blood of Case 15 at Dos de Mayo Hospital about 1½ hours after removal.

July 9. Rabbit killed and partially eaten by dogs.

Rabbit No. 35. — Testis inoculation with Oroya blood.

July 7, 1913. Right testis only inoculated with 0.5 c.c. of defibrinated blood about 1½ hours after removal from case at Dos de Mayo Hospital.

July	9.	11.50 A. M.	Temp.	101°.6 F.	Right testis and scrotum indurated and considerable oedema in scrotum. Left not found.						
"	11.	12.15 "	"	101°.9.	Right	testis	normal.	Left	not	found.	
"	15.	11.37 "			"	"	"	"	"	"	
"	18.	12.02 P.M.	"	102°.1.	"	"	"	"	"	"	
"	21.	11.44 A.M.	"	102°.3.	"	"	"	"	"	"	
Aug.	3.				"	"	"	"	"	"	

Rabbit No. 36. — Intravenous inoculation.

July 7, 1913. One cubic centimeter of defibrinated blood from case at Dos de Mayo Hospital injected into ear vein about 1½ hours after removal.

July	9.	11.45 A.M.	Temp.	99°.6 F.	Tail is soiled with faecal material. No parasites visible in the blood.						
"	11.	12.15 "	"	102°.4.	No	parasites	visible	in	the	blood.	
"	15.	11.35 "			"	"	"	"	"	"	
"	18.	12.03 P.M.	"	101°.5.	"	"	"	"	"	"	
"	21.	11.46 A.M.	"	102°.2.	"	"	"	"	"	"	

August 8. Killed. A male with small testes. Organs appear normal. No tissue saved. Blood smear made. No parasites were observed in the blood of the animal the day following the inoculation or subsequently.

Monkey No. 1. — Intravenous inoculation.

July 7, 1913. Two cubic centimeters of defibrinated blood from case at Dos de Mayo Hospital injected intravenously in vein of leg.

July	9.	11.55 A.M.	Temp.	101° F.	Slight diarrhea	Blood Smears Wright and Giemsa	Negative.
"	11.	12.25 P.M.	"	102°.	"	"	"
"	13.					"	"
"	15.	12.30 "	"	100°.9.		"	"
"	17.	10.30 A.M.	"	101°.8.		"	"
"	18.	12.25 P.M.	"	101°.9.		"	"

II. July 6, 1913. 10 c.c. of blood were drawn from vein of the arm of a rather severe case of Oroya fever (Case 8) in which the parasites (*Bartonella bacilliformis*) were demonstrable by microscopic examination in fair numbers. Animals were immediately inoculated with this blood and the protocols of the experiments are as follows:

Rabbit No. 20. — Testes inoculation with Oroya blood.

July 6, 1913. Both testes inoculated with 0.5 c.c. of defibrinated blood from Italian at San Bartolomé within six minutes after removal.

July	9.	11.15 A.M.	Temp.	101°.6 F.	Both testes normal.			
"	11.	11.45 "			"	"	"	
"	15.	10.55 "	"	101°.8.	"	"	"	
"	18.	10.43 "	"	101°.7.	"	"	"	
"	21.	11.13 "	"	101°.6.	"	"	"	
"	26.	12.13 P.M.			"	"	"	

July 28. Killed by dogs. Hemorrhage in nostrils, peritoneal cavity, and bitten in various parts of the body through the skin. Both testes appear normal. Organs present no lesions apart from those produced by biting. No tissue saved.

Rabbit No. 21. — Testes inoculation with Oroya blood.

July 6, 1913. Both testes inoculated with 0.5 c.c. of defibrinated blood from Italian at San Bartolomé within eight minutes after removal.

July	9.	11.15 A.M.	Temp.	101°.5 F.	Both testes firm.			
"	11.	11.45 "			Right testis normal; left testis homogeneous.			
"	15.	11. "	"	102°.4.	Both testes firm, normal.			
"	18.	11.40 "	"	101°.	"	"	"	"
"	21.	11.16 "	"	102°.1.	"	"	"	"
"	26.	12.13 P.M.			"	"	"	"
"	30.				"	"	"	"

Rabbit No. 22. — Testes inoculation with Oroya blood.

July 6, 1913. Both testes inoculated with 0.5 c.c. of defibrinated blood from Italian at San Bartolomé within ten minutes after removal.

July	9.	11.20 A.M.	Temp.	101°.8 F.	Both testes normal.	
"	11.	11.50 "			Testes small, firm and normal.	
"	15.	11.03 "			Testes small, firm and normal.	
"	18.	11.45 "	"	101°.5.	Testes normal.	
"	21.	11.18 "	"	102°.1.	"	"
"	26.				"	"
"	30.				"	"

Rabbit No. 23. — Intravenous inoculation with Oroya blood.

July 6, 1913. 2 c.c. of defibrinated blood from Italian at San Bartolomé injected into right ear vein.

July	9.	11.20 A.M.	Temp.	100°.6 F.	Blood smear (Giemsa) negative for parasites.
"	11.	11.50 "	"	101°.6.	Rabbit appears normal. Blood smear (Giemsa) negative for parasites.
"	15.	11.05 "	"	102°.3.	Rabbit appears normal. Blood smear negative.
"	18.	11.47 "	"	100°.5.	Blood smear (Giemsa) negative.
"	21.	11.20 "	"	102°.3.	" " " "

July 29. Dead. Yesterday noted ear which was perforated as though by a bite. At the autopsy the bladder was distended, the kidneys showed depressed red areas on the surface 1 mm. in diameter. Other organs normal. Skinned, and brain examined. No evidence of violence. Tissue in Zenker's solution. Smear stained by Wright's method; no parasites in the blood.

Conclusions.— Thus it will be seen from these experiments that we were not successful in infecting animals with *Bartonella bacilliformis*, either by intravenous or intratesticular inoculation. Intravenous inoculation of a monkey and of rabbits with large amounts of defibrinated blood from severe cases of Oroya fever did not produce any noticeable results, and the parasites observed in the blood in the cases in man were not found in the blood of the inoculated animal. Obviously if further cases for study had been at our disposal, experiments of this nature would have been continued.

On the other hand, we were able to transmit successfully the virus of verruga peruviana to rabbits, monkeys, and dogs, and to produce definite lesions in these animals. An account of these experiments is given in Section IV, pages 82–125.

Experiments in the Cultivation of Microörganisms from Blood of Oroya Fever Cases

Attempts were also made to cultivate *Bartonella bacilliformis* in vitro, and to ascertain as well if bacteria were present in the blood in uncomplicated cases of Oroya fever. On July 6, 10 c.c. of blood were drawn from an arm vein of a case of Oroya fever (Case 8), in whose blood at the time fair numbers of red blood-corpuscles infected with *Bartonella bacilliformis* were demonstrated by microscopical examination. Cultures were immediately made with portions of this blood upon the com-

mon bacteriological laboratory media and by Noguchi's method and with the media of Bass. The inoculation of the blood upon the culture media was made as follows:

3 tubes of ascitic fluid covered with paraffin oil.
1 tube of ascitic fluid (no covering of oil).
2 tubes of ascitic fluid agar + rabbit's testicle.
2 " " blood serum.
2 " " alkaline agar.
2 " " blood agar. (N N N medium.)
2 " " bile.
4 " " Bass' media.
2 " " bouillon.

All of these cultures with the exception of one of the bouillon tubes remained apparently sterile. This culture was contaminated by a spore-bearing bacillus. In the other tubes of media, during the ten days following their inoculation, microscopical examination and the gross appearances of the cultures did not reveal or suggest any growth.

On July 7 about 10 c.c. of blood were drawn from a vein of the forearm of a severe case of Oroya fever (Case 15), in whose blood large numbers of parasites (*Bartonella bacilliformis*) were present at the time. A number of cultures were made with portions of the blood as follows:

2 tubes ascitic fluid (much blood inoculated) covered with paraffin oil.
2 " " " (much blood inoculated) not covered with paraffin oil.
4 " " " agar + rabbit's testicle.
2 " " " + rabbit's testicle.
2 " bile (large amount of blood inoculated).
2 " blood agar. (N N N medium.)
3 " Bass' media.
2 flasks bouillon (large amount of blood inoculated).

All of these cultures remained sterile except as follows. In one tube of blood agar a growth developed which proved to be that of a large, thick bacillus apparently a saprophyte. One of the tubes containing ascitic fluid and blood became distinctly cloudy, but microscopical examination did not reveal the presence of any organism. In some of the cultures the red blood-corpuscles were still fairly well preserved after forty-eight hours, but no parasites could be distinguished longer in them.

On July 11 blood cultures were made again from this same case the blood being collected less than twenty-four hours before death, and inoculated upon four tubes of Bass' media, three of blood agar, two flasks of bouillon, and two tubes of bile. All of these cultures remained sterile. No distinct development of the parasites of the red cells was observed in the tubes containing blood agar or Bass' media. Cultures were also made from this case at the autopsy which occurred the following day. The culture from the heart's blood showed after twenty-four hours several large white colonies, and microscopical examination revealed a large spore-bearing bacillus. The organism was not pathogenic by intraperitoneal inoculation into two guinea pigs, and evidently represented a contamination from the air or an infection entering just prior to death. The cultures from the spleen of this case showed neither typhoid nor paratyphoid bacilli.

Unfortunately we did not have other favorable opportunities for attempting the cultivation of the organisms of the red blood-cells; nevertheless the bacteriological blood examinations of these cases of Oroya fever are important in connection with the previous studies of several investigators in relation to Carrion's fever and verruga peruviana. In considering the previous investigations which were carried on in regard to the etiology of Oroya fever and verruga peruviana in Section II attention was called to the fact that Barton, Biffi, Carbajal, Tamayo, and Gastiaburú have repeatedly isolated paratyphoid bacilli from the blood of such cases. Tamayo [1] in one of his most recent publications, analyzed thirty cases of verruga peruviana. In twenty of these paratyphoid bacilli were isolated from the blood. The organisms were not carefully identified, and although five necropsies were performed, it is stated that in three nothing special was found, and that one was negative. In the fifth the remark is made that there were a few verrugas on the spleen. However, from these bacteriological examinations, and from those of other obser-

[1] Tamayo: Crón. méd., Lima, 1905, xxii, No. 406, p. 335; No. 407, p. 349; J. Trop. Med., Lond., 1906, ix, 159.

vers, particularly of Biffi, it appears that paratyphoid infection occurs not uncommonly in some of these narrow valleys in Peru. It has been suggested before that Carrion died of paratyphoid fever and it would seem that one might be justified in the idea that Carrion's disease is really a form of paratyphoid fever, or at least has been considered as such. It is interesting to note that Eder [1] concludes that Carrion's pernicious fever is typhoid occurring in the tropics, but that he bases his conclusions particularly on Tamayo's observations.

On the other hand it is obvious that paratyphoid infection does not give rise to the rapid, pernicious anaemia observed in Oroya fever cases. This fact alone, from a clinical standpoint, refutes at once the argument that Oroya fever is a form of paratyphoid infection. Moreover, paratyphoid bacilli have not been found in our cases of uncomplicated Oroya fever infection, and even in Tamayo's cases such organisms were not isolated from all.[2] The most important fact, however, is that in Oroya fever a specific parasite is present in the red blood-corpuscles which obviously is not encountered in uncomplicated paratyphoid infections. The fact that Biffi and Carbajal [3] and Tamayo [4] claimed the isolation of paratyphoid bacilli from suppurating granulomata in cases of verruga peruviana, further complicates the question. As Darling [5] tersely remarks, "to those who have had any experience with infections by members of the typhoid-colon group in the tropics, and in view of our knowledge of the history of the relation of *Bacillus icteriodes* and *Bacillus* x of Sternberg to yellow fever, and the relation of the hog cholera bacillus to true hog cholera, which as DeSchweinitz and Dorset have shown, is due to a filterable virus, these bacteriologic

[1] Eder: J. Trop. Med., Lond., 1906, ix, 213.

[2] In our Case 15, dying from Oroya fever, no ulceration or hyperplasia of Peyer's patches was found in the intestine, but this fact would not necessarily exclude paratyphoid infection alone. In 1902 one of us (Strong, Johns Hopkins Hosp. Bull., Balt., 1902, xiii, 107) reported the first fatal case of paratyphoid infection with necropsy with the isolation of the organism from the spleen, and in this case there were no intestinal lesions.

[3] Biffi and Carbajal: Crón. méd., Lima, 1904, xxi, No. 379, p. 285.

[4] Tamayo: ibid., 1905, xxii, No. 407, p. 352.

[5] Darling: J. Am. M. Ass., Chicago, 1911, lvii, 2072.

findings of Barton and Biffi are very interesting, and they add another disease to the list of those frequently complicated by infections with bacilli of this group."

Obviously, also, in the isolation of the organisms in Lima, from so many of these cases, the technique of isolation must be considered as well as the methods employed in identifying the organisms encountered.

From this discussion, however, and that given elsewhere in Section III, it is evident that Oroya fever unquestionably has been confused with, or wrongly diagnosed as a form of paratyphoid infection, as a stage of verruga peruviana infection, and as a form of malarial fever.

Attempts to Infect and Observe Development of Bartonella Bacilliformis in Mosquitoes

On July 10 and 11 a large number of mosquitoes were allowed to bite a severe case of Oroya fever (Case 15), in the blood of which at the time many red blood-corpuscles were infected with *Bartonella bacilliformis*. The mosquitoes used in these experiments were collected as larvae from a concrete water tank at Matucana, a well known endemic center of Oroya fever. The larvae were transferred to Lima and adults were reared from them in the laboratory. The adults were placed in small cans containing sod with growing grass and covered with netting. They were allowed to bite through the net and also directly when removed from the cans and placed in test tubes. No bites were secured at first, but after a few days the insects evinced a greater desire to feed. None, however appeared to be as hungry as is usually the case with other species of mosquitoes, and none became fully engorged. Several of these mosquitoes which had imbibed blood were dissected and examined microscopically daily, the first examinations being made 48 hours after the feeding. The examinations were continued up to July 18 when all the mosquitoes had been killed and examined. No evidence of the presence of the parasites was discovered in the stomach or salivary glands and no oöcytes were observed. On account of the

minuteness of the organism in question, the negative findings in this instance should not be regarded as final or conclusive. Unfortunately we were unable to pursue these experiments further on account of not being able to obtain suitable material. The mosquitoes proved later to represent an undescribed genus and have been named *Phalangomyia debilis* by Dyar and Knab. (Plate XXXIII.) The genus is closely related to Culex as is shown by both adult and larval characters. No further specimens were taken, so that it is impossible to state whether the species is generally distributed through the Oroya fever zone, or peculiar to it. It would seem, however, that both assumptions are not unlikely. It appears very probable that Oroya fever is transmitted by the bite of some arthropod, and the demonstration of whether this fact is true should not be difficult. The fact that the parasite is present in such large numbers in the peripheral circulation naturally suggests that it is transmitted by some blood-sucking insect which serves as an intermediate host. The failure to infect either the monkey or the rabbit by inoculation of blood from a severe case of Oroya fever furnishes no support to the hypothesis that other vertebrates act as carriers of this disease. Upon our arrival at Lima we found the Peruvian government entomologist, Mr. Charles H. Townsend, engaged in the study of the transmission of verruga peruviana by insects, a subject which he had pursued for some time prior to our visit. His conclusions and results relating to this subject will be considered in that portion of the report devoted to verruga peruviana (see page 153). We regret that we did not have an opportunity to study the question of the transmission of Oroya fever either properly or completely. The question is obviously one of great importance and one which should be thoroughly and carefully pursued.

Pathology

Previous Investigations.— We have not been able to find in the literature any accurate and complete description of the pathological anatomical lesions of Oroya fever. Evidently but very few careful and complete necropsies have been per-

formed in those regions where the disease occurs endemically. Moreover, the disease often has been confused, as we have emphasized, with other diseases which occur in these localities. Both of these facts tend to explain the absence in the literature of complete and accurate descriptions of the lesions which occur in Oroya fever. In the estimable monograph of Professor Odriozola [1] we find the most complete account of the gross lesions, and Scheube,[2] Plehn,[3] and Castellani [4] also very briefly allude to the pathological anatomy of the disease.

Odriozola writing upon this subject states:

"Les lésions que l'on y rencontre sont peu considérables, eu égard a la gravité de la maladie et aux phénomènes bruyants qui l'accompagnent. Un fait anatomo-pathologique général domine l'état des divers organes, c'est l'*anémie*. Depuis la peau jusqu'aux profondeurs de l'organisme, tous les tissus sont pâles, exsangues. Néanmoins, les complications locales, congestives ou inflammatoires, ne sont pas rares et altèrent l'uniformité de cette pâleur, en quelque sorte caractéristique. Il est en effet fréquent de voir des foyers congestifs ou inflammatoires du côté des poumons, surtout aux bases; de même, on peut observer une congestion clairsemée sur la musqueuse intestinale, ou une véritable entérite.

"Le sang est très liquide, se coagule très lentement et a un aspect noirâtre. Nous en reparlerons.

"Le péricarde contient la plupart du temps une quantité de liquide séreux plus ou moins considérable. Les cavités du coeur sont vides; et on y trouve très souvent des caillots fibrineux adhérents aux parois et se prolongeant jusque dans les grands vaisseux.

"Le foie et la rate sont fréquemment engorgés. Cette hypertrophie portant sur le foie atteint parfois un volume tel que, pensant à une hépatite suppurée, on a pu faire de grossières erreurs de diagnostic. La rate peut également augmenter à tel point qu'elle descend presque jusqu'à la fosse iliaque: elle peut donner l'illusion de la cachexie paludéenne. Dans ce dernier cas il est surprenant de voir ce viscère, une fois l'éruption apparue, se réduire rapidement. Il ne faudrait pas croire que l'engorgement de ces deux organes soit constant. Nous avons constaté plusieurs exemples où la rate était d'un volume normal quelquefois même réduit. Mais il est également vrai que l'engorgement du foie est plus constant que celui de la

[1] Odriozola: loc. cit., p. 98.

[2] Scheube: Die Krankheiten der Warmen Länder, Jena, 1903, 416.

[3] Plehn: Mense, Handbuch der Tropenkrankheiten, Leipz., 1905, 1st ed., ii, 438.

[4] Castellani: Castellani & Chalmers, Manual of Tropical Medicine, Lond., 1913, 2d ed., 1201.

rate: nous l'avons enregistré dans presque toutes nos observations. La rate se présente ramollie, plus ou moins friable. Le foie offre bien souvent à la coupe le caractère ardoise, propre à l'impaludisme et qui révèle la destruction des globules rouges.

" Les ganglions lymphatiques sont totalement engorgés et cette hypertrophie dépasse celle qu'on observe dans une anémie vulgaire; ceux du mésentère surtout acquièrent parfois un volume comparable à celui que l'on trouve dans la tuberculose ou dans la leucocythémie ganglionnaire. Nous regardons l'engorgement des ganglions lymphatiques comme un excellent signe diagnostique, très appréciable aux régions accessibles, telles que la nuque, la région sus-claviculaire et carotidienne, les aisselles, les aines, etc."

Tamayo [1] has reported upon twenty-six cases of Carrion's disease, eight of which were fatal. Necropsies were performed upon five. However, almost nothing regarding the pathological conditions observed occurs in the report. In three of the cases it is stated nothing special was found at the necropsy. In the other two the autopsy findings are reported as negative with the exception of the fact that the spleen and liver were enlarged; in one a few verrugas were found upon the spleen when there had been no eruption upon the skin during life. Nevertheless, the blood counts made upon these patients during life all showed very advanced anaemia, so that some pathological changes must have been visible at the necropsies. No report upon the condition of the intestines or other organs is given.

Through the kindness of Dr. Hercelles, Professor of Pathology in Lima, we had the opportunity of examining the pathological material relating to Oroya fever and verruga peruviana in the Museum and Laboratory at the Dos de Mayo Hospital. In Dr. Hercelles' opinion the most striking lesions of Oroya fever (cases of verruga peruviana without the eruption as he designated them) are the infarctions in the spleen which occur very commonly. Infarctions in the liver he has also observed.

Present investigations. — Our ideas regarding the pathology of Oroya fever which follow, are based largely upon the study

[1] Tamayo: Crón. méd., Lima, 1905, xxii, Nos. 406, 407; J. Trop. Med., Lond., 1906, ix, 159.

of organs and tissues collected by ourselves, but the material observed in and received from this institute and from other Peruvian physicians has also been considered in our discussion of this subject. The latter material, however, will be referred to more particularly in that portion of the report dealing with the pathology of verruga peruviana.

The most striking feature observed at necropsy of patients which have died with Oroya fever is the evidence of an advanced anaemia. The skin shows marked pallor and is usually of a pale, yellowish, waxy color. There is no eruption on the skin in uncomplicated cases. The conjunctivae and finger tips are also pale. Diffuse and punctate hemorrhages may occur in the conjunctiva and in the mucosa of the nose or mouth. The superficial lymphatic glands are usually moderately enlarged, of firm consistence, and on section, of a pale pink color. Frequently they are oedematous. There is almost always considerable emaciation, and the subcutaneous fat is scanty. Small punctate hemorrhages are often observed in this tissue. A few petechial hemorrhages may occur in the epicardium, viscera, pleura, and fat elsewhere in the body. These are not extensive, however. There is a tendency to moderate oedema in many of the tissues. The heart usually contains partially fluid, pale red blood. The myocardium is often flabby; a few small discrete ecchymoses may be present in the pericardium. The lungs are usually pale. They sometimes show some congestion; more commonly, however, oedema at the base. A few petechiae are often observed beneath the pleurae, and a small amount of pleural effusion is common. The spleen is usually enlarged and of firm consistence. The follicles are not swollen and are sometimes indistinct. Infarctions are common. The liver is frequently somewhat swollen. Its consistence is flabby, and the cut section often shows yellowish mottling and sometimes areas in which necrosis and fatty degeneration is perceptible. The mesenteric lymphatic glands are swollen and on section either pale pink or somewhat reddened in color. The follicles of the small intestine may be swollen, but in other cases they may

be normal in appearance. Superficial ulcerations have been found in the large intestine. Perhaps lesions of this nature develop only late in the course of the disease. The bone marrow is usually distinctly softer than normal, and the surface sometimes shows a grayish-red mottling. Carvallo [1] has found an increased production of normoblasts and neutrophilic myelocytes, an observation which we have also been able to confirm. The necropsy of Case 15, a typical one of severe Oroya fever, the description of which was dictated at the time the necropsy was made, illustrates more in detail many of the important features in relation to the gross lesions encountered in this disease, and is therefore inserted here.

Autopsy. Case 15. — The body is that of a white male, Lithuanian, with light, dull brown hair. The body measures 1 m. 67.6 cm. and is well developed, but sparely nourished. The subcutaneous fat is very scanty. The skin of the entire body shows marked pallor, and is of a pale yellowish, waxy color. Lips and finger tips grayish white. The conjunctiva is very pale, showing numerous diffuse and a few punctate hemorrhages, and also injection of the vessels. The latter condition prevails only on the lower lids, the conjunctiva of the upper lids being also pale. Pupils distended and equal. The skin is in general entirely free from papules, nodules or tumors. There is one grayish white papule on the right forearm over the pronator radii teres muscle at its upper third, which is almost the color of the normal skin, and measures about 2 mm. in diameter; it is very slightly raised. (Preserved for histological study.) There is no other lesion noted in the skin of the entire body.

Rigor mortis is marked in both upper and lower extremities and there is moderate livor mortis over the dependent parts; consisting of pale red blotches on the dorsal aspect of the trunk. The body is still warm. The autopsy is commenced two hours after death.

On stripping the pectoral muscles there is practically no visible subcutaneous fat. The muscles are moist, and are only slightly paler than normal. The subcutaneous and intermuscular fat over the left inguinal region shows small punctate hemorrhages from mere points to 1 mm. in diameter. The inguinal lymph-nodes are somewhat enlarged, of firm consistence and of a pale pink color. The marrow removed from the femur two inches above the median portion appears distinctly softer than normal, and is for the most part of a dark red color; the cut surface shows a slight mottling, with gray in certain areas.

On opening the abdominal cavity the peritoneum is smooth and shows a slight yellowish tinge over the surface of the intestines. The appendix is long and bent upon itself behind the ileocecal valve. The most notable abnormal feature is the condition of the spleen, to be described later on.

The pleural cavities contain a small amount of fluid. There are no adhesions. The pericardial sac contains a small amount of pale, straw-colored fluid, possibly slightly increased in amount. The epicardium presents numerous small discrete

[1] Carvallo: Thèse de Lima, 1911.

ecchymoses, varying from mere points to 1 mm. in diameter. These are most numerous in the region of the apex, and over the posterior surface of the heart.

The heart is rather large and contains a small amount of soft clot, but much pale red blood, which distends the chambers. The heart valves appear practically normal, although the tricuspid presents several soft yellowish thickenings at the border. Above the aortic valve are small whitish elevated areas somewhat irregular, and often connected with one another. The intima of the coronaries is smooth. Beneath the endocardium of the left ventricle there are opaque areas of dull grayish color, which are both discrete and confluent, and give the surface a mottled appearance. In the right auricle there are a few discrete opaque spots from 1 to 3 mm. in diameter. The myocardium is somewhat flabby, and of a pale yellowish brown color. The wall of the left ventricle is approximately 1.5 to 1.8 cm. in thickness.

Lungs. — The left lung contains air throughout, and there are no adhesions or palpable nodules. The anterior portion, including nearly the whole of the upper lobe, is of a pink gray color, mottled with a small amount of black pigmentation, while the remainder of the lung presents a great number of small petechiae of light red color, from mere points to 2 or 3 mm. in diameter. These are most numerous over the dorsal or dependent surface. The contiguous borders of the lobes show a small amount of gelatinous material, possibly exudate. In the diaphragmatic surface are scattered numerous minute elevations of dull grayish color, not perfectly rounded, but somewhat irregular, with a smooth surface. On section the cut surface reveals no macroscopical lesions. The right lung contains air throughout, and presents practically the same features as the left, the petechiae being distributed chiefly over the dorsal surface. The apices of both lungs are free from nodules or thickenings. The peribronchial lymph-nodes are not notably enlarged or reddened on section, although somewhat juicy.

Spleen. — Capsule smooth and tense; the surface in general shows bluish color, with irregular patches of grayish pink beneath the capsule. The organ measures 17.7 × 10.1 × 7.6 centimeters. On section the cut surface appears dark reddish purple, with areas of dull brown. The surface is slightly uneven except in several areas to be described. The follicles are not visible. The consistence is firm, and on scraping the cut surface only a small amount of pulp is obtained. There are a number of infarctions, having a dry, yellowish gray appearance. None of these are distinctly wedge-shaped, although the diameter is always considerably greater at the surface of the spleen than in the spleen substance. A few are roughly triangular in shape, but one is oval. They measure from 2–3 mm. to 3–4 cm. at the periphery of the spleen.

The Liver. — Measures 27.9 cm. × 19 cm × 6.3 cm. Capsule is smooth and glistening, with a few irregular scar-like indentations on the anterior surface in the region of the gall bladder. The entire surface shows whitish translucent markings of a branching character, as though marking the course of vessels containing pale reddish blood. The cut surface of the liver substance presents distinct markings which are translucent at the periphery, so that a net work of pale glistening translucent tissue is apparent: included within this is a yellowish zone, with a dull red center. The consistence of the liver is rather flabby, so that it feels soft to the hand, but it is slightly tough and cuts with some difficulty.

The *gall bladder* contains golden olive-colored bile. The *pancreas* is very pale throughout, and presents no lesions.

There is a chain of enlarged *lymph-nodes* running the length of the pancreas, and situated at the superior border. These are rounded, dull pink in color, with lobular markings. On section they are found to be rather soft and oedematous. The

largest is situated at the tail of the pancreas, and is red in color, this appearance evidently resulting from hemorrhage.

The *kidneys* are rather large for the size of the individual. The capsules strip readily, leaving a smooth surface. Both kidneys are of a yellowish gray color with very pale pyramids and papillae. The cortex is of normal thickness; glomeruli barely distinguishable. There are no hemorrhages or other lesions present. The *adrenals* are thin, and present no abnormal feature.

The *ureters* are normal. The *bladder* contains about 150 c.c. of urine. The mucous membrane is very pale, almost white. In the anterior wall are two ill-defined thickenings which on section show no definite lesion. The *prostate* is small, and on section appears of almost homogeneous consistence, and of a whitish gray color. The *testes* show no lesions.

The *stomach* contains about 250 c.c. of thin reddish brown fluid. The mucous membrane is a dull grayish color, with slight reddening in several places.

The small intestine shows no macroscopial lesions. The solitary follicles are not swollen. The mucosa of the *large intestine* is generally gray in color. There are very numerous superficial ulcers, which are brownish red in color, and measure 3 or 4 mm. in diameter. Some have reddened, irregular borders, others are round, and with slightly undermined edges. There are a few *Trichocephalus trichiurus* in the cecum.

The mesocolic lymph-nodes are slightly swollen and pink in color.

The ulcerations noted above, scattered throughout the large intestine, are evidently in the healing stage.

The *aorta* throughout its length, as far down as the renal veins, shows whitish irregular elevations of a reticulated outline.

The head. — The scalp is relatively thick, and the skull is from 7 to 9 mm. in thickness. The cranium is of a rather broad, rounded form. The dura is nowhere adherent to the calvarium, and presents nothing abnormal, with the exception of a lenticular mass of pinkish gray color, elevated above the external surface, measuring 2.5 to 3 mm. in diameter.

The blood-vessels in the surface of the brain are filled with pale red blood. The surface of the brain is rather moist, and the pia is somewhat oedematous. The convolutions are not flattened. On removing the brain there is considerable fluid around the base, and escaping from around the cord. This is of a clear pale yellow color. The lateral ventricles contain only a small amount, about 5 c.c., of yellow fluid. The choroid plexus is pale yellow and gelatinous.

Anatomical Diagnosis.— Oroya Fever. Anaemia. Petechial hemorrhages in epicardium, viscera, pleura, and inguinal fat. Ulcerations large intestine. Slight acute pleuritis with effusion. Acute splenic hypertrophy. Infarctions of spleen. Hyperplasia and congestion of lymph-nodes. Active bone marrow. Cloudy swelling of kidney. Myocarditis. Endarteritis chiefly of aorta. Oedema of pia.

Microscopical Examination of the mucus from the intestinal ulcers did not reveal any amoebae or other animal parasites. A few ova of *Trichocephalus trichiurus* were found in the intestinal contents. The bacteriological examination of the cadaver is considered on page 39, and the microscopical examination of smears from the lymphatic glands and spleen on page 33.

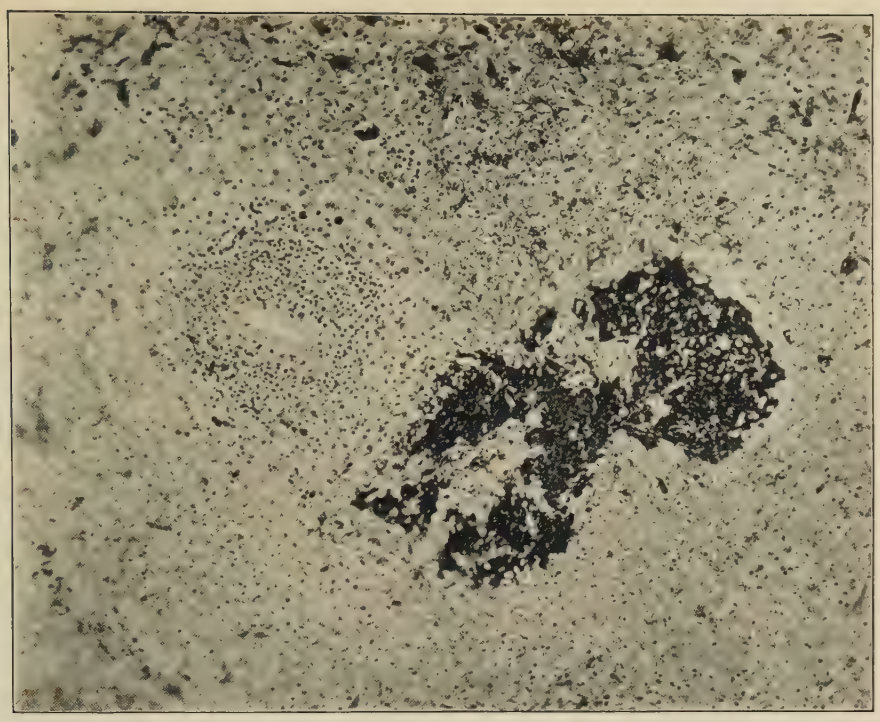


Fig. 1. — Spleen. Area of necrosis at border of malpighian nodule.

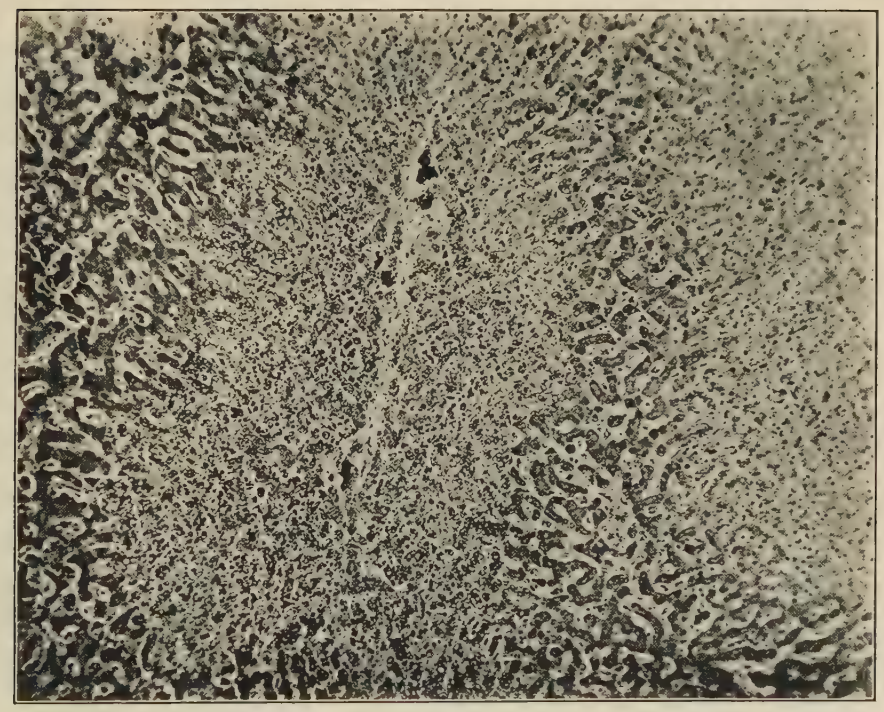


Fig. 2. — Liver. Showing area of necrosis, central type.

Plate XII. — Oroya Fever.

Fig. 1. — Liver. Stained with Scharlach Roth. Showing areas of central necrosis.

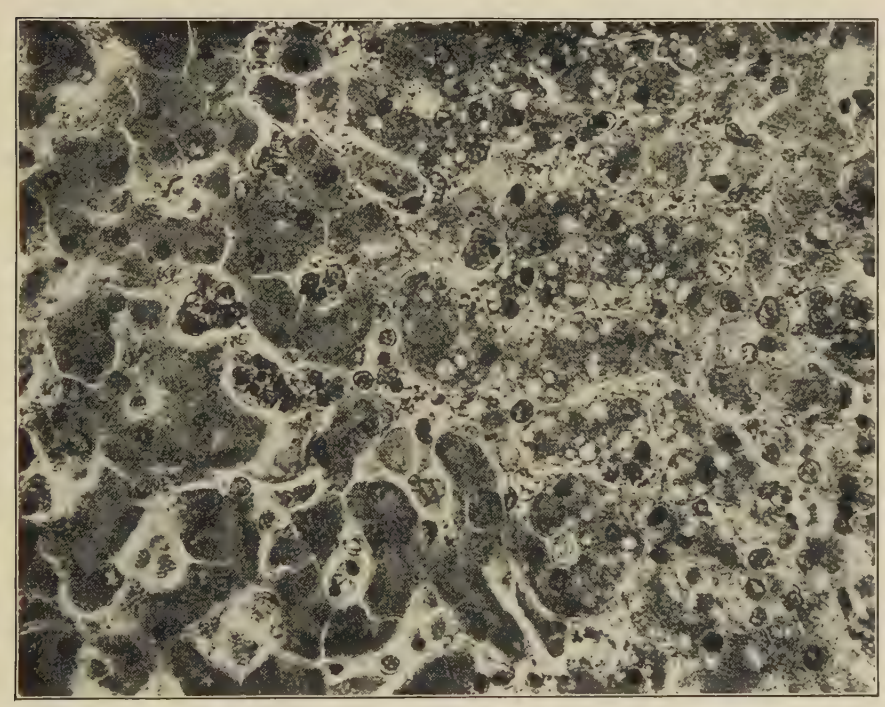


Fig. 2. — Liver. Showing area of necrosis. Fatty degeneration of liver cells and phagocytosis.

Plate XIII. — Oroya Fever.

Histopathology

We have also been unable to find any accurate report of the histological changes occurring in Oroya fever, or indeed any account of the specific lesions we have observed in the microscopical study of the tissues of individuals dying from this disease. Nevertheless, in tissues presented to us in Lima from Oroya fever cases (designated as verruga cases in which there was no eruption upon the skin), we have sometimes found the same lesions we have observed in the tissues collected at necropsy by ourselves. The tendency, by many Peruvian physicians at least, has been to regard the case coming to necropsy with no lesions of verruga either upon the skin or internally, as one of mistaken diagnosis or as one of verruga peruviana in which death has occurred before the nodular lesions have appeared. The interest in Peru has appeared almost always to center upon the histology of the verrugas.

Present investigations. In the histological study of the tissues from persons dying from Oroya fever we have found marked changes in the liver, spleen, bone marrow, and lymphatic glands.

The liver shows areas of toxic degeneration which have apparently resulted from the activity of the parasite *Bartonella bacilliformis*, and this fact would suggest that during the course of the disease in addition to the destruction of the red cells by the parasites developing in them another pathological process results from the presence of a toxin in the circulating blood. The action of such a toxin seems to be revealed in the liver by the presence of extensive areas of necrosis of the central type, beginning about the hepatic veins. (Plate XII, Fig. 2.) In these areas many of the liver cells in sections hardened in Zenker's solution show numerous vacuoles in their cytoplasm which suggest spaces formerly occupied by fat, and in tissues hardened in formalin, frozen when cut, and stained with *scharlach Roth*, the characteristic stain for fat is obtained in these areas. Distinct zones of these cells containing fat droplets surround central areas of more marked necrosis in which many of the liver cells are very granular.

Other liver cells about these areas appear homogeneous and hyaline and stain intensely with eosin. (Plate XIII.) Much granular material is present in the sinusoids and numerous swollen endothelial cells and endothelial phagocytes frequently containing erythrocytes, erythroblasts, and polymorphonuclear leukocytes are present in the periphery of the necrotic areas between the liver cells. (Plate XVI, Fig. 1.) While there is not extensive pigmentation of the liver, moderate amounts of pigment in granules or small masses may be seen within the endothelial phagocytes and in a few endothelial cells lining the sinusoids. (Plate XIV, Fig. 2.) Occasionally small granules of pigment may be seen in the liver cells. Most of this pigment does not give the iron reaction; it is yellowish or brownish in color, and not black. Crystals of hematoidin are also present.

The *spleen* shows numerous infarctions. In sections the edges of the infarcted areas are much congested and show leukocytes migrating into the necrotic tissue. Many of the veins show thrombosis suggesting endothelial injury from some toxic or infectious agent. At the periphery of many of the splenic nodules just outside the capsule, areas of necrosis are visible in which there is marked deposition of fibrin. (Plate XII, Fig. 1.) There is a large amount of pigment in the spleen, which occurs in small or larger masses and also in fine granules. (Plate XV.) It is yellowish or yellowish-brown in color, and is present in both the endothelial leukocytes and also free in masses between the splenic cells. The pigment is not black, and does not resemble black malarial pigment. It resembles melanin in that it does not give the iron reaction. Whether in Oroya fever the anaemia partially results from the destruction of the red blood-cells through the circulation of hematin, as W. H. Brown [1] believes to be the case in malaria, at the present time is not clear. *Bartonella bacilliformis* evidently produces no pigmentation in its development in the red cells in the peripheral circulation. No malarial parasites are visible in the splenic sections in uncomplicated cases. The spleen is rich

[1] Brown: J. Exper. Med., 1913, xviii, 96.

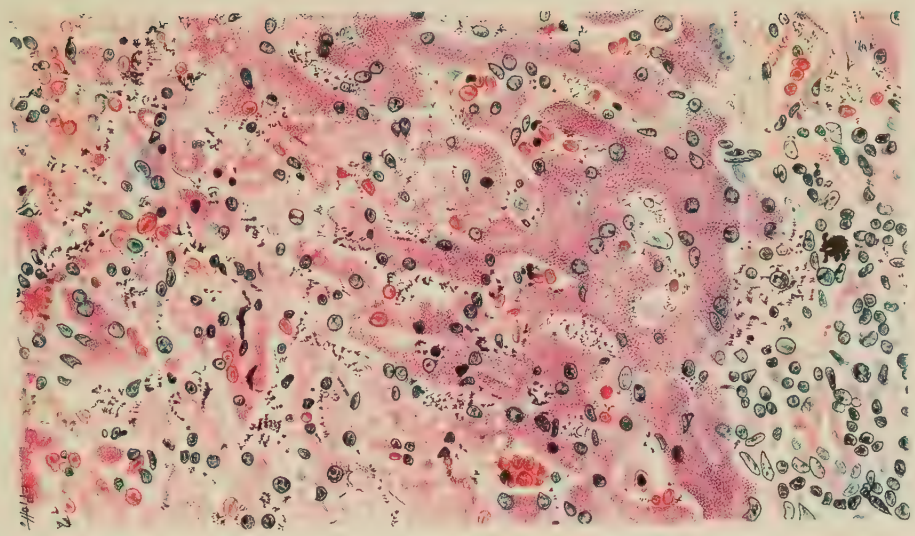


Fig. 1

Showing necrosis and the character of pigment.

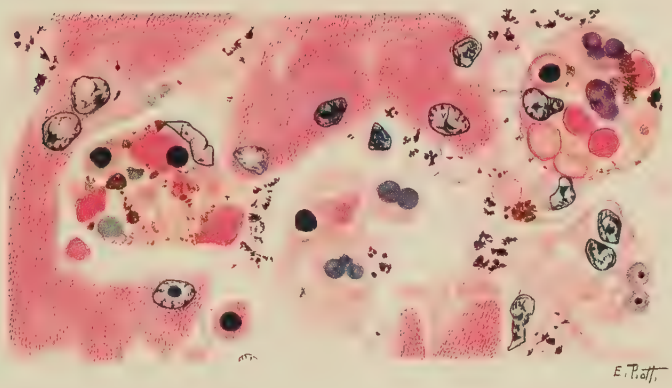


Fig. 2

Showing endothelial phagocytes in sinusoids, enclosing erythrocytes, erythroblasts, polymorphonuclear leukocytes, and pigment.

PLATE XIV — OROYA FEVER LIVER

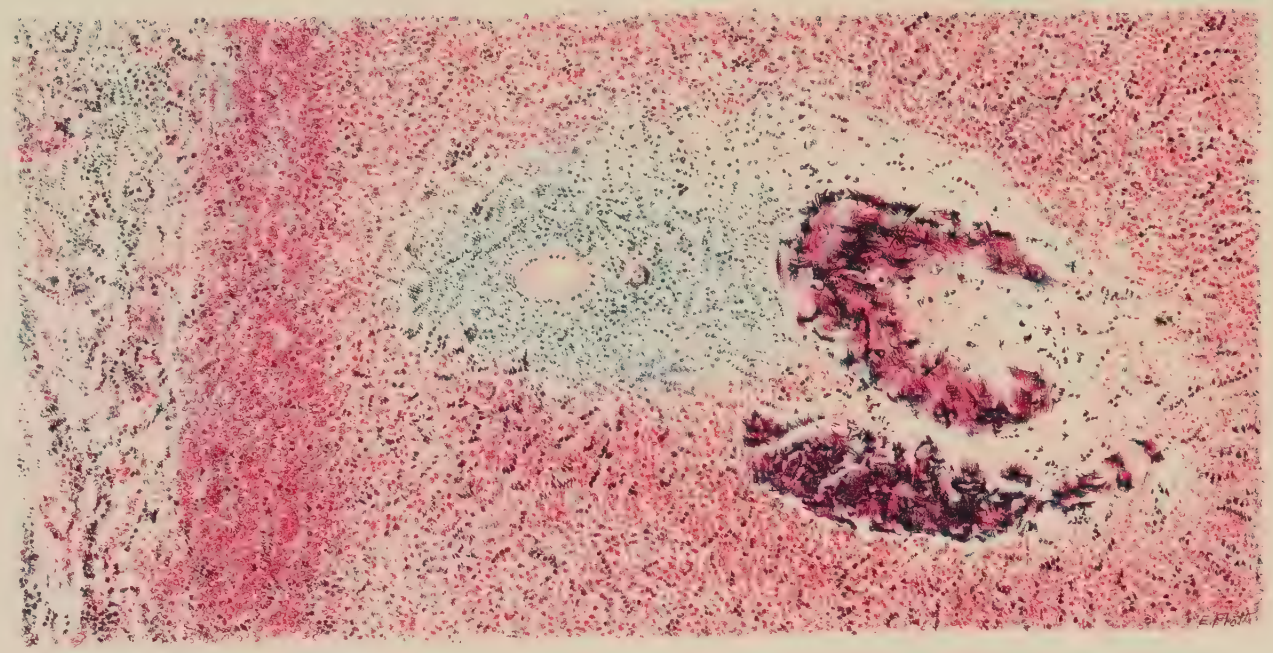


PLATE XV — OROYA FEVER SPLEEN

showing fibrin deposition and character of pigment.

in pulp and much congested; many nucleated red blood-corpuscles are visible. Large swollen endothelial cells, sometimes free, at others attached to the walls of the blood-vessels, and occasionally containing rounded and rod-shaped elements are observed. Endothelial phagocytes frequently containing red blood-cells are also present in abundance. In the *bone marrow* there is also striking evidence of phagocytosis by endothelial leukocytes of the red corpuscles in various stages of development, and of polymorphonuclear leukocytes as well as increased production of normoblasts. It seems probable that this extensive phagocytosis which is evident in the liver, spleen, and bone marrow is also an indication of the action of a toxic substance which has led to chemical changes or injury in the engulfed cells.

In the *lymyhatic glands* the most striking pathological picture which at once attracts the attention is the presence of large, swollen, endothelial cells, sometimes free in the lumen of the blood-vessel, at other times still attached to the walls. (Plates VIII and IX.) In some instances a single endothelial cell is so swollen that it will almost occlude the entire lumen of the vessel. (Plate XVI, Fig. 2.) These cells are in various stages of degeneration. Many of them are distended with rounded and rod-shaped elements and it is apparent that a multiplicative phase in the development of the parasite *Bartonella bacilliformis* takes place within these cells. (Plates VI and X.) The details regarding the cycle of this parasite and the changes it undergoes in these cells already have been referred to on page 32.

The *large intestine* shows ulcerations with distinct undermining of the mucosa at the edges so that frequently the lesion has the appearance of a small abscess cavity lying in the submucosa. There is a more or less distinct line of demarcation of the abscess with vascular granulation tissue forming the wall; numerous polymorphonuclear leukocytes, fibrin, necrotic tissue and bacteria are present. In some places coagulation necrosis is marked. (Plate XI, Fig. 2.) No amoebae or other animal parasites are visible nor does the

ulcer show anything histologically characteristic of any other disease. The blood-vessels in the vicinity of the lesions show endothelial cells distended with the rounded and rod-shaped parasites already described. These are also found elsewhere in the blood-vessels of the submucosa in small numbers and in the lymphoid tissue of the submucosa they are present in large numbers where they sometimes appear to obstruct the blood-vessels. It appears probable that this may be the primary factor in the production of the ulcerations, and that they are brought about in a somewhat similar manner to the ulcerations of the intestine sometimes produced by *Leishmania donovani* in kala-azar infection. The sections of the other organs reveal no changes of importance.

We have said that we have been unable to find any accurate account of the histopathology of uncomplicated cases of Oroya fever in the literature. In an instance in which sections of the spleen were sent to Bindo De Vecchi,[1] somewhat similar lesions were evidently encountered by him.

> In the case from which the sections came there were no nodules; also the clinical history and the autopsy made no mention of such condition. The hemorrhagic lesions of the spleen were very striking. These began in the follicles and finally destroyed them. The liver was so markedly changed that the tissue could hardly be recognized. The sections showed so-called " dissociation of the lobules " to such a degree that the characteristics of the cells were no longer present, but they were massed together. The single cells showed different lesions of degeneration or necrosis of the cell. Also different liver cells showed in their protoplasm either single or multiple inclusions of round bodies that stained deeply with a basic stain, with a ring about them, and often accompanied by precipitated pigment. In the markedly changed tissue there were also found small recent hemorrhages and a rich infiltration of leukocytes. The endothelial cells were swollen or desquamated, and some of them assumed the function of phagocytes. De Vecchi believed that the inclusions of protoplasm in the liver cells were to be regarded in all probability as the remains of nuclei from leukocytes, and that they were to be explained through the phagocytic function taken over by the liver cells as occurs in the severe degenerative lesions of the liver, and particularly in acute yellow atrophy.

The tissues upon which these descriptions of De Vecchi are based, however, were sent to him from Peru, and he had been informed that the patient from which they came had died of severe Carrion's fever. It also was stated that no verrugas of the skin or internal organs were observed at the necropsy,

[1] De Vecchi: Beiheft 4, Arch. f. Schiffs- u. Tropenhyg., Leipz., 1909, xiii, 143.

Fig. 1. — Liver. Showing endothelial phagocytes in sinusoid.

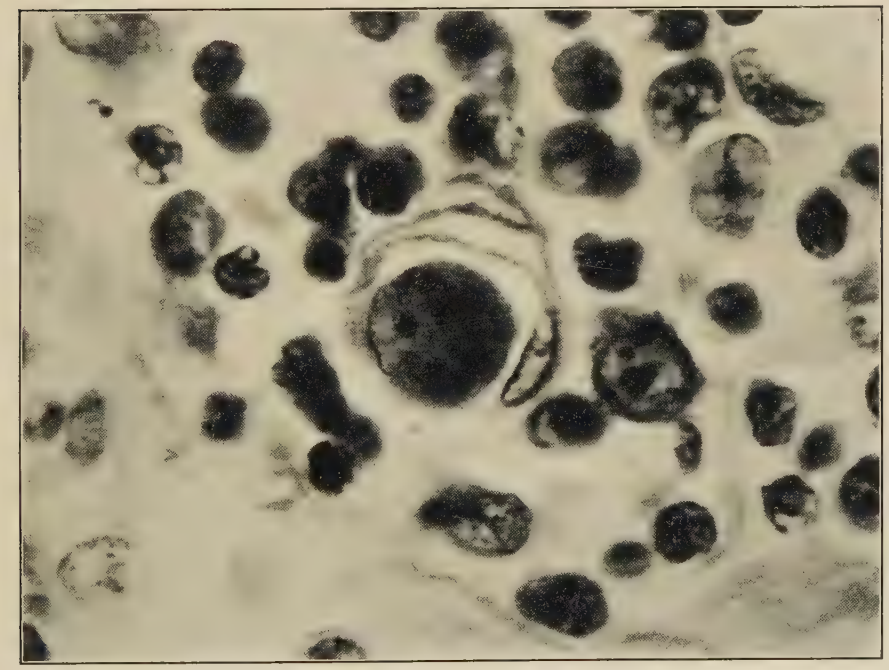


Fig. 2. — Lymph-node. Showing swollen endothelial cell in blood-vessel wall.

Plate XVI. — Oroya Fever.

and that a paratyphoid bacillus had been isolated from the spleen. Possibly the organism encountered in this case was a species of paracolon or colon bacillus, and a terminal invader in a case of Oroya fever. In the light of our own observations the changes described in the sections of the liver by De Vecchi suggest such a possibility.

Histological Examination of other Tissues

Just prior to our departure from Lima, and after our investigations there had been completed, we obtained from the Hygienic Laboratory and from the Dos de Mayo Hospital blocks of tissue from necropsies not performed by ourselves. In these cases a diagnosis of Oroya fever or of verruga peruviana had been made during life. We were unable to obtain complete histories of these cases. In some it was stated there had been verrugas upon the skin during life, or in the viscera at autopsy. However, our experience in Peru has led us to believe that lesions may be sometimes referred to as having been due to verruga peruviana which more careful investigation showed to be of an entirely different nature. These tissues have been sectioned and studied, and the notes made regarding them are as follows:

Histological Examination of Material from other Cases in which a Diagnosis of Oroya Fever or of Verruga Peruviana was made by Peruvian Physicians

Case A. Clinical Diagnosis, Oroya Fever.

Liver. — Shows uniformly distributed central degeneration with vacuoles in the liver cells probably representing fat. An occasional phagocyte situated in the sinusoids presents a few golden-yellow pigment granules. The endothelial cells of the small vessels occasionally show the parasites which have been previously described fully in this report. (See page 33.) These are not prominent features and are only found after considerable search. They are more numerous in the small vessels of the sinusoids near the periphery of the section where the preservation is more perfect.

Spleen.—Follicles atrophied. Several show relatively large areas of fibrinoid material at periphery. Spleen is without pigment. Infected endothelial cells are present but found only on careful search.

Lymph-Nodes. — Show no marked pathological change apart from the swelling of the endothelial cells of the blood-vessels which are distended with small rod-shaped bodies. These changes are found more frequently in the small veins.

Bone Marrow. — Small fragments of bone marrow consist of about one-half fat. There is considerable phagocytosis but no notable lesion. No characteristic changes are found in the endothelial cells.

Remarks.—This is undoubtedly a true case of Oroya fever from the characteristic degeneration of the liver and from the presence of *Bartonella bacilliformis* in the liver, spleen, and lymph-node. Note that there is practically no pigment present.

Case B. Clinical Diagnosis, Oroya Fever. (Formalin fixation.)

Liver. — Shows a large amount of golden-brown and dark pigment distributed in phagocytes situated in sinusoids. There are numerous focal lesions consistent in character with miliary tubercles, and acid-fast bacilli are found.

Spleen. — Lymphoid tissue present in considerable amount. Small amount of yellowish-brown pigment. Focal lesions showing acid-fast bacilli.

Remarks. — There is no certainty that this is a case of Oroya fever. Diagnosis: — General miliary tuberculosis; presence of malarial pigment questionable.

Case E. Clinical Diagnosis, Oroya Fever.

Liver. — Extensive central degeneration with trabeculae replaced by spaces filled with blood; at the periphery in such areas there are the remains of necrotic liver cells, still further out the liver cells are for the most part vacuolated. Occasional small granules of pigment are found in phagocytic cells.

Lymph-Node. — A mass of inflammatory tissue with interior portion necrotic. There are giant-cells at the periphery and smaller foci characteristic of tuberculosis. A section specially stained; shows no acid-fast bacilli.

Bits of brain tissue present no unusual feature.

Two large lesions consisting of very vascular inflammatory tissue.

Remarks. — This case is more or less indefinite in several respects. The liver is interesting in that it suggests a later stage of Oroya fever, possibly one bordering on repair, since the degenerated parenchyma has completely disappeared in the central portion of lobules. The gland is probably tuberculous and the vascular inflammatory tissue is probably from a verruga lesion.

Case W. Clinical Diagnosis, Oroya Fever. (Preservation poor.)

Liver. — Generalized central necrosis with vacuolation of surrounding liver cells. Small amount of pigment — large numbers of coarse bacilli distributed generally throughout the tissue.

Spleen. — Shows a small amount of pigment. Finer changes not possible to distinguish.

Remarks. — This case shows central degeneration of the liver, and a small amount of pigment in this organ and the spleen. Possibly the tissues are from a case of Oroya fever, but there is no positive evidence of this from the histological appearances.

Case X. Clinical Diagnosis, Verruga and Oroya Fever. Viscera fixed in formalin.

Liver. — Shows focal lesions consistent with tuberculosis, also a considerable amount of golden-brown pigment within phagocytes.

Spleen. — Also shows focal lesions containing acid-fast bacilli. There is a small amount of pigment.

A Lymph-Node. — Shows tuberculosis but no changes in the endothelium such as those found in Oroya fever. Contains peculiar rounded, oval, or long dumb-bell-shaped bodies included within phagocytes. These vary from reddish purple to almost black.

Skin. — Very vascular tissue covered by epithelium consistent with verruga.

Remarks. — General miliary tuberculosis, possibly verruga lesions of the skin. No positive evidence of Oroya fever. Presence of malarial pigment questionable. Peculiar oval bodies in a tuberculous lymph-node.

Case Y. Clinical Diagnosis, VERRUGA, OROYA FEVER, AND MALARIA. Preserved in sublimate, July, 1912.

Liver. — Considerable phagocytosis, very little pigment. Bacilli distributed throughout organ. No marked degeneration.

Spleen. — Practically no lymphoid tissue in evidence. No pigment.

Remarks. — The amount of material is scanty; there is no evidence in the sections confirmatory of the diagnoses furnished for the case.

Case Z. Clinical Diagnosis, VERRUGA PERUVIANA.

Liver. — Poorly preserved; almost impossible to distinguish any pathological process.

Spleen. — Much phagocytosis and occasional pigment granules.

The bone marrow shows no pathological change.

The Skin Lesions. — Are composed of vascular tissue covered over with epidermis. Some show multiple hemorrhages.

Remarks. — There is enough to identify the latter lesions as those of verruga peruviana, but the preservation of the tissue is such that it is impossible to form any conclusions as to the existence of Oroya fever in this case.

Material obtained at the Dos de Mayo Hospital from cases diagnosed verruga peruviana

Specimen from Heart. — Shows multiple cysts. Sections show that these are of a parasitic nature and are of the nature of cysticercus cysts (probably *C. cellulosae*). No scolices apparent. Three sections examined.

Specimen from Pharynx. — Situated immediately beneath and partially destroying the epithelium are numerous rounded masses of vascular tissue. There are also large masses extending somewhat deeper. There is no tendency to caseation and no giant-cells. Lesions show no acid-fast bacilli in specially stained sections.

Remarks. — These lesions resemble those of verruga peruviana.

Spleen. — Several areas of degeneration at the periphery of lymph follicles. There is considerable dark brown pigment which from its distribution suggests artefact. There are, however, a few granules within the phagocytic cells. No characteristic changes found within endothelial cells, although preservation is poor.

The results of the study of these tissues are interesting. From the description we have already given in this report of the pathology of Oroya fever, it is evident that the histology of this disease is pathognomonic; and we see in the study of the tissues described above of at least one of the cases, that a definite diagnosis of Oroya fever could be made from the presence of these characteristic lesions, in which the swollen endothelial cells containing the parasites were present. As has been stated, the diagnosis of some of these cases given to us was " verruga peruviana, with Oroya or Carrion's fever."

It is evident that cases of verruga peruviana have been mistaken for cases of tuberculosis. This question will not be considered here, but will be discussed more fully elsewhere in this report in relation to the disease verruga peruviana. (See page 138.) Here it is merely desirable to call attention to the fact that the tissues of Cases B and X evidently came from patients suffering with tuberculosis. It seems evident that the tubercles present in the liver in these cases were considered as verrugas of the miliary type. That other such mistakes have been made we are confident from the fact that miliary tubercles in the spleen and liver due to *Bacillus tuberculosis* have been shown us by physicians as internal verrugas. It therefore is clear that in Peru miliary tuberculosis has sometimes been diagnosed incorrectly not only as verruga peruviana but also as Oroya fever. Obviously however miliary tuberculosis may occur as a complication of either disease.

The distension of the endothelial cells of the blood-vessels of lymph-nodes, spleen, and liver of Case A, and the presence of large numbers of the parasites demonstrate that these tissues came from a case of Oroya fever and confirm the clinical diagnosis of the disease in this instance.

IV

VERRUGA PERUVIANA

Geographical Distribution

The history of the disease already has been considered in Section II. From this account it is evident that verruga peruviana has been recognized from remote, historical periods in Peru, and gradually its geographical distribution has become well defined. So far as is known the disease is confined to South America. It is certainly more commonly found on the western slopes of the Andes in Peru. However it seems likely that cases also occur in southern Ecuador, parts of Bolivia, and the northern part of Chili. This is the view held by Firth.[1] Zárate in 1545 refers to the occurrence of the disease upon the Ecuadorian coast, and Hall [2] reported a case in which the infection was contracted in Zaruma, Ecuador. Formerly it was stated its distribution was limited in the regions in Peru between 9° and 16° south latitude. Today it is usually said to be confined between 6° and 13° south latitude. It seems possible, however, that verruga peruviana is related to, if not identical with, the affection described by Bassewitz [3] as "angiofibroma cutis circumscriptum contagiosum" which he found to occur in southern Brazil. If this is the case the distribution of verruga peruviana must be still further extended to 20° south latitude. Rocha-Lima [4] following the suggestion of Bassewitz states that upon clinical grounds verruga peruviana and angiofibroma contagiosum are probably different diseases, since in verruga peruviana the eruption is preceded by an acute febrile stage, and the mortal-

[1] Firth: Allbutt and Rolleston, System of Medicine, 1912, ii, Part 2, 704.

[2] Hall: Lancet, Lond., 1883, Part 2, 845.

[3] Bassewitz: Arch. f. Schiffs- u. Tropenhyg., Leipz., 1906, x, 201.

[4] Rocha-Lima: Verhandl. d. deutsch. pathol. Gesellsch., 1913, April, 409, Gustav Fisher, Jena.

ity is much higher. Rocha-Lima has evidently accepted the idea that Oroya fever and verruga peruviana are different stages of the same disease. Since he has apparently never seen a case of Oroya fever and but one case of verruga peruviana, and since we have found that the mortality in uncomplicated cases of verruga peruviana is very low, and that the fever preceding the eruption is rarely severe or dangerous, the clinical manifestations, urged by Rocha-Lima as distinguishing features, between verruga peruviana and angiofibroma contagiosum are not apparent. However, further investigation is necessary before one can decide definitely upon the identity of these conditions. So far extensive inoculation experiments in animals with the angiofibroma contagiosum have not been performed.

Today the most important endemic areas of verruga peruviana appear to be located in the narrow valleys on the western slopes of the Peruvian Andes, locally known as *quebradas*, situated at an altitude of from 2,800 to 9,000 feet. (Plates XVII and XVIII.) In these regions the disease often becomes epidemic. In the wider valleys in the vicinity it seems to be less prevalent. The localities particularly affected have in general the same natural features. There are deeply cleft, narrow valleys whose sides are formed of comparatively bare rock, consisting largely of granite and diorite, and are almost barren of vegetable growth. Owing to the absence of rain during the greater part of the year, the extremely rocky and barren character of the soil, and the great intensity of the sun's rays during the day, that vegetation which occurs upon the slopes consists of extremely xerophytic types. Conspicuous among these are cacti of several kinds including species of Opuntia and Cereus, particularly the peculiar white-hooded *Cereus senilis*. (Plate XVIII.) A fleshy Euphorbia with cylindrical, jointed and branched stem, resembling an Opuntia is common among the cacti with which it might be confused except for its thin, cylindrical, deciduous leaves. A specimen brought to Boston later developed the characteristic leaves of Euphorbia. A few grasses occur on the higher slopes but all are dry and parched in the drier season of the year.

Fig. 1. — Regions in the Andes Mountains in which Oroya Fever, Verruga Peruviana, and Malaria are prevalent.

Fig. 2. — View down the Rio Rimac from above Matucana, showing the town.

Plate XVII. — Peru.

Fig. 1. — Type of Vegetation on the lower slopes of the Cañon close to the River along the Trail from Surco to Matucana.

Fig. 2. — Type of Vegetation covering the higher slopes of the Cañon along the Trail from Surco to Matucana.

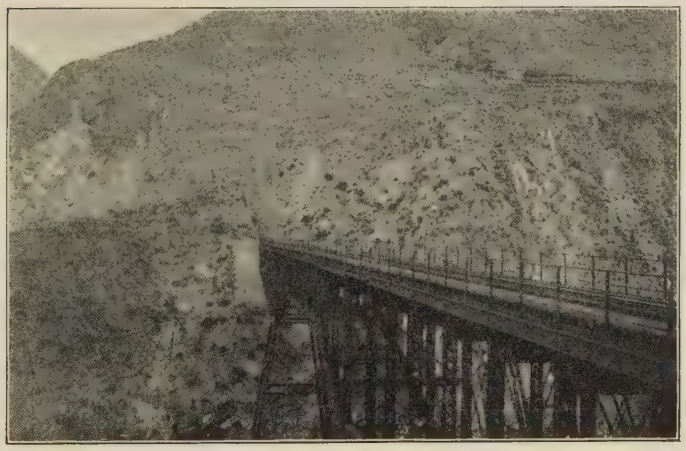


Fig. 3. — Oroya Fever Bridge in the Andes Mountains.

Plate XVIII. — Peru.

Trees are almost entirely absent, although a very few scattered specimens may be seen here and there far up the slopes. Cold mountain streams run through these valleys, the soil of which is formed particularly of clay which is covered with luxuriant vegetation in strong contrast to that on the slopes. Odriozola has given a most interesting account of the flora in these regions. The vegetation, however, is abundant only in the vicinity of the streams or rivers, or where irrigation ditches have been constructed, since, as intimated, practically very little rain falls in these regions the greater part of the year. Here and there, water is drawn from the rivers by means of sluiceways and carried some distance down the valley where it is used to irrigate the fields that occupy the nearly level valley between the steeply rising mountains on each side. As the rivers descend rapidly over numerous cascades, plunging at times among enormous boulders and again flowing over short gravelly or rocky stretches, there is little vegetation along the immediate edge of the banks. The irrigated fields, however, are extremely fertile and numerous cattle find good grazing here in patches of grass and alfalfa. Just above these there is a semi-arid level with numerous plants; agaves, leguminous trees resembling Mimosa, Prosopis and Parkinsonia, and a great variety of flowering herbs. Maize and potatoes are grown to some extent in the irrigated areas, but no other crops except forage plants are extensively cultivated. Toward the lower limit of the verruga zone, a few fruits are cultivated such as oranges, peaches, and even apples in addition to the usual tropical fruits like the cherimoya, granadilla, avocado pear, etc., but these are characteristic of the lower level rather than of the verruga zone. Along the course of the river and also up the mountain slopes on the edges of the smaller streams that rush down the mountain side, there is a large amount of pampas grass (*Gynerium argenteum*) which species appears to be quite characteristic of the verruga zone. Likewise, the Prosopis-like trees and Mimosas are absent from the lower levels where the place of the pampas grass is taken by its larger congener, *Gynerium gigan-*

teum. It would thus appear that the verruga zones have, by reason probably of their altitude, a flora quite noticeably different from Chosica and the other towns just below the region where the disease occurs.

The climatic conditions in these valleys are also peculiar and different from those of Peru in general. In contrast to the rainless coast of Peru, there is what might be called a rainy season here, even though the rainfall is so scanty. The disease appears to be most prevalent in the summer, and particularly in the months of March and April when the mountain streams or rivers are in flood, that is, about the close of the rainy season. The days in these valleys are hot and sunny, and the temperature may reach as high as 95° to 103° F., but the sun sets behind the high mountains early in the afternoon, and the nights are cold and during them the direction of the wind is usually reversed. A diurnal variation of from 25° to 35° F., is not infrequently observed. Odriozola, in his monograph on verruga peruviana, gives the geographical distribution in Peru up to 1906 as follows:

> In the Department of Lima there are six provinces of which four, Yauyos, Huarochirí, Canta and Chancay, constitute endemic areas of the disease. These provinces contain six separate river systems, and include nineteen infected communities, and seven additional known infected but uninhabited localities. The altitude in these regions ranges between 2,900 and 7,900 feet. In the Ancachs Department the provinces of Cajatambo, Huaráz, Huaylas and Pallasca, the last two extending into the southern edge of Libertad Department, containing four separate river systems, are also infected, showing eight isolated valleys, with sixteen infected communities, the altitude of these districts being from 1,300 to 10,900 feet.

Townsend [1] states that the Magdalena quebrada, in the department of Cajamarca, has very recently been found to be a strong focus of verruga. A number of other infected localities have been discovered since the publication of Dr. Odriozola's monograph and further investigation has shown the disease to be present in the departments of Lima, Ancachs, Libertad, Lambayeque, Cajamarca, probably the southeastern corner of Piura, and perhaps the southwestern part of Amazonas.

[1] Townsend: J. of Economic Entomology, Phila., 1913, vi, 224.

It is not deemed advisable or necessary to enumerate in this report the names of all the towns and villages in Peru where the disease is known to occur. During our visit to Peru the most seriously infected areas were those situated in the vicinity of the railway extending from Lima to Oroya, in the territory between Chosica with an altitude of 2,800 feet and Matucana with an altitude of 7,789 feet. The towns particularly infected at this time were Santa Eulalia, San Bartolomé, Surco, Cocochacra and Matucana, and the cases we studied were discovered largely in these towns or their vicinity, or in the hospitals in Lima. The cases we observed in Lima were said to have contracted the disease in these districts. In the map given by Professor Odriozola in his monograph, Matucana is not included in the endemic areas. Cuesta Blanca with an altitude of 6,177 feet, and Surco with an altitude of 6,660 feet, are the points with the highest altitudes given as endemic centers. This fact shows that the location of these centers varies somewhat from year to year. We were informed that the disease appeared first in Matucana in epidemic form in March and April, 1912. As mentioned, we also found this locality infected in 1913.

Etiology

Castellani and Chalmers, writing in 1913 in their "Manual of Tropical Medicine," say the causation of verruga peruviana is quite unknown, and this is the opinion generally accepted and which is expressed in our other modern textbooks upon tropical medicine and in the literature generally relating to this disease. By the natives verruga peruviana for a long time was supposed to be contracted by drinking water, and this view was accepted by Tschudi [1] who claimed that if one avoided drinking from the suspected springs "aguas de verrugas" they escaped the disease. Dounon [2] however, first showed that there was no basis for this theory, and he and his assistants all drank from the so-called infected springs and

[1] Tschudi: loc. cit. [2] Dounon: loc. cit.

streams without contracting the disease. Raimondi [1] thought that the poison of the malady might originate from venomous batrachia which live in the water, or that the disease might be due to a virus as rabies or syphilis. In 1875 Pancorvo [2] called attention to the fact that the persons who usually contracted it worked in the fields or were brought in some way into intimate contact with the soil, and he suggested that it was an intoxication due to sulphureted hydrogen liberated from the earth. This view never received marked support, but Firth [3] in 1912 suggested that the true solution of the pathology and etiology of this affection will be found in the association of the disease with some form of parasitic worm whose free stage is passed either in water or mud, most likely in mud. Tupper [4] suggested that the disease was transmitted by a miasmatic agent. Chastang [5] called attention to the fact that many observers consider the thorns of the *Cactus opuntia* to be the cause of the disease, because the pricks from this plant produce cutaneous lesions. He suggests that parasites clinging to the thorns may be the cause.

An account of the microörganisms which previously have been described as the etiological factor of verruga peruviana has already been given in Section II, Page 10, and they need not be referred to in detail here. As may be seen from this account, Izquierdo, Florez, Odriozola, Barton, Biffi, Tamayo, Gastiaburú, Nicolle, Letulle, Escomel, Galli Valerio, Mayer, Rocha-Lima, and Werner have all described various microörganisms as the cause of the disease. In 1913 [6] we were able to show that verruga peruviana is due to a specific virus which may be transmitted definitely to man and to several species of the lower animals by direct inoculation. The details relating to these facts will be referred to more at length in the pages which are to follow.

[1] Raimondi: El Perú, 1874.
[2] Pancorvo: Gac. méd., Lima, 1875, 167.
[3] Firth: Allbutt and Rolleston, System of Medicine, 1912, ii, Part 2, 705.
[4] Tupper: Ueber die Verruga Peruviana, Inaug.-Diss., Berlin, 1877.
[5] Chastang: Arch. de méd. nav., 1897, 417.
[6] J. Am. M. Ass., Chicago, 1913, lxi, 1713.

It is generally stated in the textbooks that the disease is not contagious, and in this statement we agree in that it certainly does not appear to be markedly contagious, since in cases treated in the general wards of hospitals no tendency is observed for it to spread. It is also generally stated that the disease is transmitted by direct inoculation, and that this was proven by Carrion's experiment. Although we have the highest admiration for the courage of Carrion, we do not believe that the experiment which he performed, in which he inoculated himself with the blood from a verruga nodule and died subsequently with fever, demonstrated that the disease verruga peruviana is inoculable. No lesions developed upon the skin in Carrion's case according to the reports, but no one can tell today definitely of what he died, since there is not dependable and sufficient data upon the subject. As mentioned above, however, it has since been shown that the virus of the disease may be directly transmitted from man to man, with the production of definite lesions and without fatal result.

Age, sex, and *race* apparently have no influence upon the occurrence of verruga peruviana. We observed a number of cases in children of varying ages, and in both male and female adults. We saw cases in young nursing children, and Campodonico [1] and Monge [2] state it occurs in newly born infants. Nearly every one who lives in the infected areas acquires the disease and then becomes immune. One attack usually protects. In those cases in which reinfection occurs the disease appears in mild form. Accession or relapses, however, during the course of the malady are not uncommon. Strangers on visiting and remaining for any length of time in the endemic areas usually become attacked. It has been repeatedly stated in the literature that it is only necessary for a non-immune person to spend a single night or to sleep in an infected district in order to acquire the disease. In regard to many statements of this nature, it should be borne in mind that they have often been made in reference to Oroya or Carrion's fever. Firth [3]

[1] Campodonico: Crón méd., Lima, 1895, Feb. 15.
[2] Monge: J. Lond. School Trop. Med., 1912, Vol. i, Part 2, 164.
[3] Firth: loc. cit.

points out that prolonged residence in the endemic centers is certainly not necessary to contract the disease, although a mere passage through the country without either eating or drinking on the journey, or being thrown in intimate contact with the inhabitants is not sufficient to produce it.

Clinical Features

Incubation Period.— Castellani states the incubation period of the disease is not definitely known, that it has been variously estimated to be from eight to forty days, but is most usually from twenty to thirty days. Odriozola, with the widest clinical experience, gives the incubation period from fourteen to forty days. Firth's views coincide with this, and we have no evidence to offer to the contrary. We should be inclined to regard the average period as from about fourteen to twenty-one days. In one human case experimentally inoculated the period which ensued between the inoculation and the appearance of lesions was sixteen days. In monkeys according to our experiments the incubation period usually varies from eleven to twenty-two days; in one experiment of Jadassohn [1] it was sixty days.

Symptoms.— It is generally stated in textbooks of tropical medicine that there are three clinical stages of verruga peruviana: (1) of invasion; (2) of eruption; and (3) of recovery. The stage of invasion in our cases was usually characterized by pain in the joints, the knees, elbows, ankles, and wrists being more commonly affected, and by moderate fever. The temperature sometimes may reach 104° F., but more often does not exceed 100°.4 F. The fever in our experience ushers in the eruption and is usually of short duration, lasting sometimes but a few days. Other observers say the fever may last a much longer time. Castellani gives from twenty days to eight months; Firth from one to nine months or a year. Odriozola gives the average period of fever from three to four months. Malaria is such a very common disease in the endemic areas of verruga peruviana, and so many of the inhabitants of these

[1] Jadassohn and Seiffert: Ztschr. f. Hyg. u. Infectionskrankh, Leipz., 1910, lxvi, 247.

Fig. 1. — Early Miliary Eruption.

Fig. 2. — Miliary Eruption. More Advanced Stage.

Plate XIX. — Verruga Peruviana

Fig. 3. — Eruption. Most Active Stage of Disease.

Fig. 4. — Eruption. Beginning to Recede

(From Lumiere Plates).

Fig. 1 — Nodular, Subcutaneous, and Mulaire Lesions.

Fig. 2. — Nodular amd Mulaire Lesions.

Plate XX. — Verruga Peruviana (From Lumiere Plates).

districts are infected with malaria that it is perhaps not strange that these statements regarding the fever vary so much. Thus Scheube [1] says malaria is an almost inseparable concomitant of verruga and bears a certain relation to the disease. Plehn [2] also refers to the frequent association with malaria and states that the fever in verruga is doubtless sometimes due to malaria. Hercelles and Gastiaburú found many verruga cases infected with malarial parasites. Patients with typhoid fever and Oroya fever in these districts would also be very likely to contract verruga. Many Peruvian physicians, among them Odriozola, admit that in the cases of verruga peruviana not preceded by the Oroya fever stage, the fever preceding the eruption is of a brief period and may not be severe. Castellani says slight cases may pass through the whole illness without fever, while more severe ones may show intermittent or remittent fever; this is also our experience in relation to the fever in this disease. Following the initial fever the eruption appears upon the skin and the temperature may become normal, or a slight evening rise, of a degree or two, may persist for a varying length of time. Sometimes during the period of invasion, particularly at the onset, there is complaint of some headache and backache. The physical examination of the chest usually reveals nothing abnormal. The spleen and liver are not enlarged in uncomplicated cases. The eruption upon the skin which is the characteristic feature of the disease occurs in a variety of forms according to the stage of the infection.

Eruption.— The cutaneous lesions have been termed "miliary," "nodular," or "mulaire," according to their size and mode of development. The eruption consists at first of erythematous areas in which groups of small, pin-point sized papules soon form. Small hemorrhagic vesicles also often appear which may be grayish or reddish, or dark blue, according to their degree of vascularity and the character of the blood within them. (Plate XIX.) The areas over which this so-called "miliary" rash appears are frequently oedematous, and this

[1] Scheube: The Diseases of Warm Countries, Lond., 1903, 2d ed., p. 299.
[2] Plehn: Mense, Tropenkrankheiten, 1905, ii, 435.

is most commonly observed on the legs, and more rarely upon the extensor surface of the arms. Unless the disease is arrested the papules grow until they usually measure from 1 to about 5 mm. in diameter. They are generally reddened, raised, and sharply circumscribed from the surrounding tissue, more often discrete, sometimes confluent, and during the most active process of the disease, are of a bright or dark cherry color. The skin over them is tense, translucent, and adherent. (Plate XIX.) They are at this time usually firm or cushiony to the touch. When injured they bleed freely. Later in the disease they assume a gray color or become the color of the skin. As the disease advances, in addition to the cutaneous papules deeper subcutaneous nodules may appear, which at first are frequently free from the skin and lie deep in the subcutaneous tissue. Later many of these grow towards the surface and the skin over them becomes involved and adherent. (Plate XX.) These nodules vary from several millimeters to three or four centimeters in diameter. The nodular type is especially common about the flexures of the knees and elbows and over the thighs and legs. After these nodules have become adherent to the skin, the skin over them sometimes becomes broken and they may ulcerate, reaching the surface as red fungating masses. These lesions on becoming protuberant are known as verrugas of the "mulaire" type and often measure several centimeters in diameter. (Plates XX, and XXI.) They also may be pedunculated. While the eruption occurs most commonly on the legs and arms, particularly the extensor surfaces, and over the shoulders, it not infrequently appears on the face, forehead, and ears. Sometimes it appears on the trunk (Plate XXIII) and sometimes upon the palms of the hands and soles of the feet. A better idea of the extent and distribution of the lesions can be obtained from the illustrations. (Plates XIX to XXIII.) The miliary eruption also may occur in the mucous membrane of the mouth or nose, or upon the glans penis. Sometimes when situated in the nose epistaxis may result. After lasting for several weeks and sometimes for three or four months or even longer, during which time the eruption and nodules may

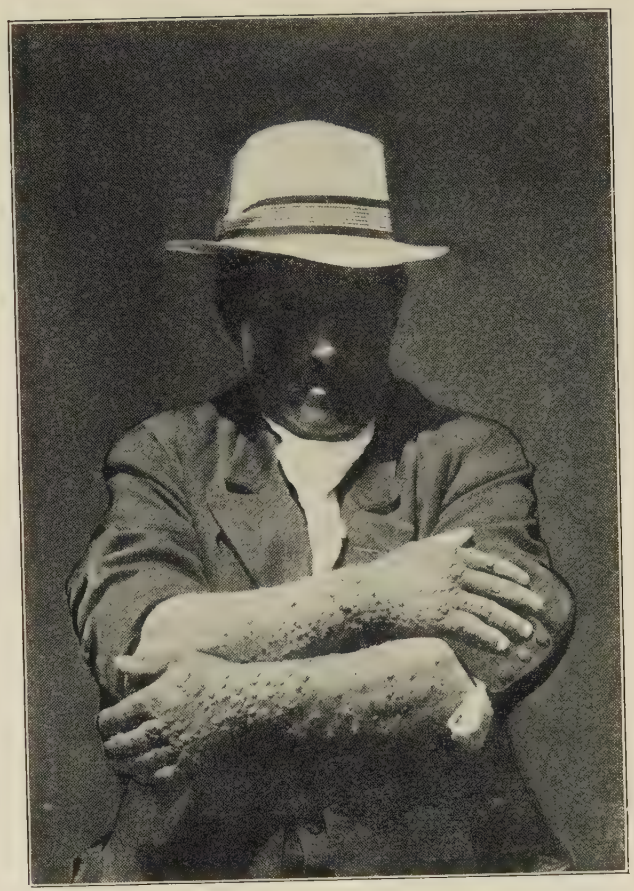


Fig. 1. — Acute stage of disease.

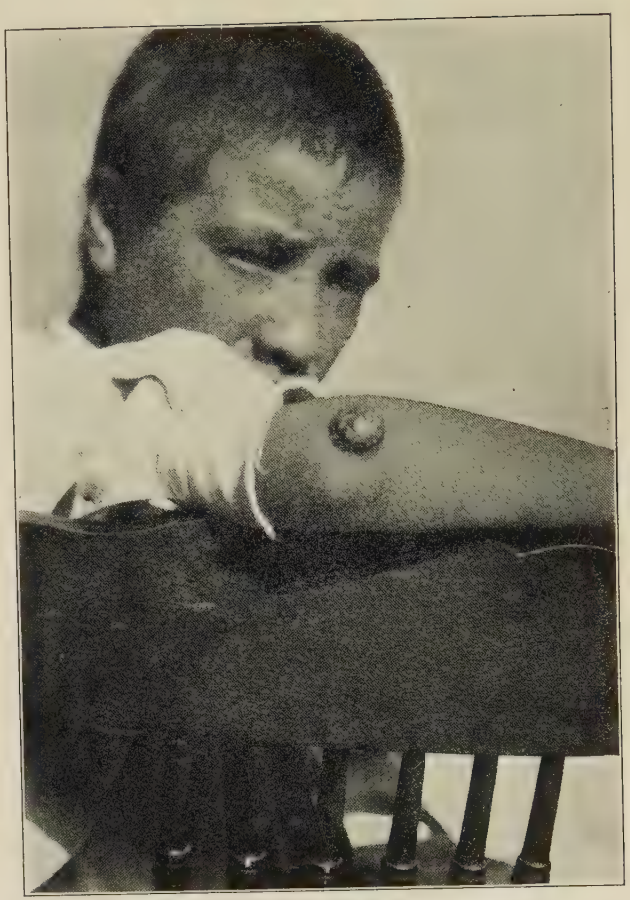


Fig. 2. — Advanced mulaire lesion.

Plate XXI. — Verruga Peruviana.

Fig. 1. — Showing nodular and subcutaneous lesions.

Fig. 2. — Showing nodular lesions.

Plate XXII. — Verruga Peruviana.

disappear and reappear, that is, accessions or relapses occur, the eruption fades, dries up, and the nodules become absorbed without leaving a scar. The ulcerating mulaire lesions heal by cicatrization. In some cases only the miliary eruption appears and convalescence supervenes after several weeks.

The opinion is often expressed that the eruption is often found in the internal organs, and that there exists no mucous membrane or organ in which it may not appear, and various symptoms have been ascribed to its presence in the internal organs. We have been unable to find sufficiently definite evidence for these general conclusions, and it appears to be one of those statements that has succeeded from one author to another until it has become generally accepted. One does find in the early literature statements regarding the occurrence of the verrugas in all parts of the body, but many of these statements should be accepted with caution, since an accurate knowledge of pathology had not at that time become very widely disseminated, and many incorrect diagnoses of lesions were obviously made. Certainly the pathological material preserved in Lima relating to this disease does not give this idea of the distribution of the lesions. The subject will be more fully discussed when the pathology of the disease is considered.

Blood. — Since many if not the majority of cases of verruga have previously suffered from malaria, it is not unusual to find anaemia present, but no such advanced anaemia exists as has been observed in cases of Oroya fever, and the parasite of Oroya fever is not found in the blood-corpuscles in uncomplicated cases. Monge [1] states one finds a marked anaemia, but that enormous differences occur in the number of the red blood-corpuscles. According to him a characteristic feature of the disease is a diminution of the signs of the anaemia from the time the patient enters the hospital. In our experience in well marked and severe cases of the disease the red blood-corpuscles did not number usually less than three million. At the onset of the eruption the leukocytes are sometimes

[1] Monge: J. Lond. School Trop. Med., 1912, i, Part 3, 239.

increased, but usually during the course of the disease they are normal or reduced in number. Monge calls attention to the fact that there is a definite mononuclear increase during one stage of verruga. The following table shows the number of red and white cells and the hemoglobin estimation in several severe cases of the disease in the eruptive stage.

Case 1.	Red blood-corpuscles	3,900,000
	White blood-corpuscles	14,000
	Hemoglobin	70 % (Sahli)
	Red blood-corpuscles	3,000,000
	White blood-corpuscles	8,400
	Hemoglobin	75 % (Sahli)
Case 2.	Red blood-corpuscles	3,000,000
	White blood-corpuscles	4,400
	Hemoglobin	50 % (Sahli)
Case 3.	Red blood-corpuscles	3,800,000
	White blood-corpuscles	22,600
	Hemoglobin	55 % (Sahli)
Case 16.	Red blood-corpuscles	3,000,000
	White blood-corpuscles	4,200
	Hemoglobin	65 % (Sahli)
Case 17.	Red blood-corpuscles	2,300,000
	White blood-corpuscles	4,300
	Hemoglobin	55 % (Sahli)
Case 18.	Red blood-corpuscles	1,600,000
	White blood-corpuscles	4,500
	Hemoglobin	45 % (Sahli)
Case 19.	Red blood-corpuscles	3,300,000
	White blood-corpuscles	6,000
	Hemoglobin	45 % (Sahli)

The differential counts on these cases were strikingly different from those upon Oroya fever (see page 20). The following illustrations are taken from two advanced cases of verruga; in one the white count was normal, while in the other a leukocytosis was present. In both the percentage of mononuclear cells is increased, whereas the myelocytes of Oroya fever are absent.

	Case 1	Case 2
Polymorphonuclear neutrophils	47 %	45 %
Large mononuclears	15	9
Transitionals	8	4
Lymphocytes	27	39
Eosinophils	3	(2 cells)
Unclassified	—	2

PLATE XXIII. — VERRUGA PERUVIANA. Showing general distribution of nodular lesions.

Prognosis.— It is usually stated that the mortality of verruga peruviana varies between 10 and 40 per cent, but this evidently includes cases complicated with other diseases. In our opinion verruga peruviana when uncomplicated with other diseases is very rarely fatal.

Cases illustrating Special Features

The histories of the following cases selected from the protocols of the cases studied by us will serve to emphasize many of the clinical characteristics of the disease.

Case 1. — *Extensive Eruption; miliary, nodular, and mulaire lesions.* June 18, 1913. Seen in hospital in Lima, aged eighteen years, lives at Chosica, but has recently worked for long periods in Santa Eulalia. Had tertian malaria six months ago. Present illness of three months' duration. No fever noticed at onset. No verruga among family or friends, but he has been associated with several cases in his work. Patient is sitting on bed, looks anxious, slightly dyspnoeic.

Head.— Eyes equal, movements good in all directions, sclerae clear. Pupils equal, react to light and accommodation. Mucous membranes very pale. Ears are free from tophi and discharge. Teeth are rather well preserved. Tongue very slightly coated. Tonsils not enlarged. Neck is somewhat thin. Posterior cervical glands are not palpable. Both lobes of thyroid are palpable, but isthmus is not felt. There is slight fulness over the right parotid, but there is no tenderness on pressure. Patient complains of sensitiveness and pain on pressure just under the ramus of the left maxilla.

Both epitrochlear glands are markedly enlarged. They would apparently measure about 1.5 to 2 cm., and are somewhat sensitive on pressure.

Chest is well formed and rather deep. Respirations are shallow,—thirty to the minute. Respiratory movements equal on both sides. Vocal fremitus better felt in right subclavicular region than on the left. Percussion note slightly dull over right subclavicular region but otherwise equal over fronts and axillae. Breath sounds somewhat harsh over right supra- and infraclavicular regions. No râles heard. Fronts and axillae otherwise clear.

Heart.— Point of maximum impulse best seen and felt at mamilla. No thrills nor shocks. Relative cardiac dulness extends 1 cm. to right of sternum, and 2 cm. to left of midclavicular line. Sounds very distant but clear at apex. Not heard over aortic and pulmonic areas.

Abdomen.— Soft, hepatic and splenic dulness is not increased, and neither the liver nor spleen is palpable.

Extremities.— Deep reflexes are active. Romberg negative.
Blood examination:

June 22,	Wassermann reaction negative to beef heart antigen.	
June 27,	Red blood-corpuscles	3,900,000
	White blood-corpuscles	14,400
	Hemoglobin	70 % (Sahli)
July 19,	Red blood-corpuscles	3,000,000
	White blood-corpuscles	8,400
	Hemoglobin	75 % (Sahli)
July 22,	Wassermann negative to four antigens.	

The examination of the blood reveals no parasites. The urine is normal. There is an extensive eruption upon the skin.

Cutaneous Lesions.— The cutaneous lesions are extensive and comprise lesions of the miliary, nodular, and mulaire types.

Distribution. — Lesions occur over the extensor surfaces of hands, forearms, and arms. (Palmar surface of hands and flexor surface of forearms and upper arms clear.) Forehead, particularly above right eyebrow, cheeks, and over malar regions. A few pin-point to pin-head sized vesicles over lips, ears, shoulders, and over both scapulae. Back and trunk generally entirely free from lesions. Dorsal surfaces of both feet, over ankles, anterior surface of the tibiae, and right thigh. Also on the posterior surface of the left thigh.

Description of Lesions.— Right Arm, Forearm.— Entire flexor surface of the right arm, as well as that of the palm of the hand, is entirely free from lesions. On the extensor surface of the hand, at the base of the first and second fingers, over the articulation between the metacarpal bones, are groups of small, discrete, and confluent papules, some whitish and others pinkish red in color, measuring usually about 1 mm. in diameter, and slightly raised above the surface of the skin. They are hard to the touch and have a rough feeling when the finger is rubbed over them. Over the wrist there is a papule measuring 1.5 mm. in diameter, and raised about 1 mm. above the surrounding skin. It has a dome-like appearance, is translucent, and pink in color. It is sharply circumscribed from the surrounding skin. Scattered over the extensor surface of the whole right arm are very numerous papules, varying from pin-point to about 2 mm. in size. The smallest papules are grayish white in color, and just visibly raised above the surface of the skin; those that are more slightly raised and slightly larger are quite hard to the touch. The lesions which are slightly larger are from pink to bluish red in color. Some are the exact shade of cherries. These are translucent in appearance, are dome-like in character, sharply circumscribed from the surrounding skin and discrete. They appear very much as a cherry in regard to translucence; that is, they suggest a softer consistence beneath their perfectly smooth capsular membrane. This last description refers to those areas which are about

2 mm. in diameter. They are raised for about 1.5 mm. above the surrounding skin, and are quite hard to the touch. Upon pressure of some of the pinkish lesions they become grayish in color, and evidently a great deal of the fluid can be pressed out of them. Upon pressure on others, however, the cherry-like appearance is not lost. Some of the smaller lesions appear almost vesicular. A few have become hemorrhagic and appear as dark red points on the skin, measuring about 1 mm. in diameter, and very slightly raised.

Over the bursa of the right elbow is a nodular area measuring 2 cm. in diameter, and about 1 cm. in height. It is firm to the touch and appears to be situated outside of the bursa.

Over the back of the arm, and on its exterior aspect, are very numerous papules, similar to those described upon the forearm. A number of them, however, are dark purple or black in color. The largest, however, does not measure more than 2 mm. The darker areas evidently represent a more advanced stage of the lesions. The progression seems to be from the small gray pin-point sized lesions to the pink and cherry-colored ones; then from the resulting hemorrhages the lesions become dark purple or black, and finally dry up, leaving blackish scabs. Beneath some of the blackish scabs, however, some nodular tumors apparently sometimes form. Upon the right arm, over the insertion of the deltoid into the humerus is a small nodular tumor of this character measuring 5 mm. in diameter, and about 1 mm. in height. It evidently represents a small verruga of the nodular character. The lesions over the right shoulder are similar to those described on the arm and forearm.

Left Arm, Forearm, and Hand.— Flexor surface and palm of hand entirely free from lesions, with the exception of one lesion situated on the wrist at the base of the thumb. This lesion measures 13 mm. in diameter and about 4 mm. in height. It is moderately firm to the touch, and not freely movable beneath the skin. Its color is brownish pink.

Over the thumb there are two nodules measuring each 12 mm. in diameter, and having a height of about 5 mm. They are sharply circumscribed and quite firm to the touch. They are not freely movable beneath the skin. They are almost the color of the surrounding skin, but slightly redder.

Situated over the third finger, at the distal end of the metacarpal bone is a granulating area 2 × 1.5 cm. from which a tumor has been removed for study.

Over the wrist, forearm and arm are numerous miliary lesions similar to those described on the right arm, but they are not so numerous. Over the upper third of the arm, on its posterior surface is a small tumor measuring 5 mm. in diameter and about 1 mm. in height, which is grayish blue in color, sharply circumscribed and moderately firm to the touch. When

pressed it indents slightly, suggesting that its contents have been somewhat absorbed.

Face.— Over the forehead there are scattered very numerous grayish and pinkish miliary lesions. Over the right eyebrow are a group of discrete and confluent pinkish and cherry-colored papules, measuring usually about 2 mm. in diameter. Over both cheeks there are numerous miliary nodules, one on the right cheek having reached a diameter of a little over 2 mm., and a height of 1 mm. Over the right inferior maxilla is a nodular tumor measuring 4 mm. in diameter and 2 mm. high. It is sharply circumscribed, moderately firm to the touch, grayish white in color, in the center and at the edges grayish brown. On the conchae of both ears are numerous lesions of the miliary type, and similar to those already described. They are much more marked in the right ear. The left ear contains only fifteen or twenty of these lesions. Just anteriorly to the left ear is a small nodule measuring 5 mm. × 3 mm., and about 1.5 mm. in height, of the nodular type.

Right Leg.— Over the anterior surface of the right leg and foot are a number of lesions, all of the nodular type. No miliary lesions are anywhere visible on the leg and foot. The nodular lesions measure from 6 mm. to 1 cm. in diameter. Some are brownish in color, others brownish red, and some have a pinkish tinge. There is one measuring 1 cm. in diameter midway over the third metatarsal bone. Over the right toe is a granulating area 1.5 cm. in diameter from which a tumor has been removed. There are two tumors measuring 1 cm. in diameter, one over the external and one over the internal malleoli, and there are four more small tumors over the anterior surface of the tibia. Two of these seem to be drying up. Over the anterior surface of the tibia 7 cm. above the ankle joint is a lesion of the so-called mulaire type. Its border is irregular in outline, it is soft to the touch, and fluctuates slightly. Evidently there is some fluid deep in the subcutaneous tissue. The lesion measures about 2 cm. in diameter, but it is not sharply circumscribed, and its exact diameter cannot be stated.

Over the right thigh above the knee is a group of several hundred lesions of the miliary type, and one of the nodular type, measuring 1 cm. in diameter, and 2 mm. in height; this is quite firm to the touch, and the skin over it has become dry and whitish gray in color. The tumor itself beneath the skin appears of a dull pink color. Posteriorly over the soleus muscle is a small tumor of the nodular type about 6 mm. in diameter. It is the only lesion on the posterior surface of the leg or thigh. The sole of the foot is clear.

Left Leg.— On the anterior surface of the thigh there are numerous lesions of the miliary type. All of the nodular type are below the left knee. These consist of seven tumors measuring from 5 mm. to about 12 mm. in diameter. Several have been removed for section. These are all sharply

circumscribed. One has reached a height of nearly half a centimeter above the surrounding skin. Over most of them the skin has become dried and grayish white. Just below the external malleolus is a fungating mushroom-like tumor with a pedicle. It measures 2 cm. in diameter at the top, and is raised for a distance of 1 cm. above the surrounding skin. The pedicle has a diameter of about 12 mm. The surface is in places grayish and yellowish in color, and in others reddish. This variegated appearance has resulted from the erosions. This lesion is of the mulaire type. On the leg there is posteriorly one nodular area over the middle of the soleus muscle; six over the anterior surface of the leg, and one over the dorsal surface of the foot. Over the middle of the tibia is another mulaire lesion measuring about 3 cm. in diameter, and not sharply circumscribed. In the center there is slight fluctuation. The edges are firmer to the touch. The sole of the foot is clear.

Laboratory Examination. — Many microscopical preparations, both fresh and stained, were made from the lesions of the skin during the several weeks that the case was under observation. Many of the fresh preparations were examined with the dark-field microscope. No spirochaeta or other recognizable microörganism was found present either by microscopical examination or by animal inoculation. Attention has been called to the fact that the Wassermann reaction was negative. Numerous cultures and animal inoculations were made from this case. The notes regarding these experiments will be found on pages 82 to 105.

The patient under generally tonic treatment gradually improved. No fever was noted. The lesions became slowly and completely absorbed, and after about five weeks' stay in the hospital he left the institution apparently well.

Case 17.— Papular, cutaneous, and subcutaneous nodular eruption.— July 10, 1913, Male, aged eighteen years. This patient gives a very indefinite and unreliable history. He lives in Lima but worked in Cocochacra two days in February, 1912. He has had malaria twice, once in childhood and again beginning June 23, 1913. He had a short attack of verruga fever in August, 1912, lasting two weeks. The present eruption followed the attack of malaria and rheumatism in June of the present year. He does not know of any association with verruga patients.

Examination.— Conjunctivae are pale in color. The cervical glands are not felt; the left epitrochlear gland is about 2 cm. in diameter. Two glands are palpable in the right epitrochlear region each about 1 cm. in diameter. The inguinal glands are very large and firm. From the history given they were probably involved during an infection with Ducrey's bacillus about eighteen months ago. The examination of the heart and lungs reveals nothing abnormal. The liver is not enlarged. The spleen is firm and

extends about 5 cm. below the costal margin. The urine is normal. The blood shows no parasites. A count gives:

Red blood-corpuscles	2,300,000
White blood-corpuscles	4,300
Hemoglobin	55 % (Sahli)

The Wassermann reaction is negative.

There is an extensive eruption upon the skin.

Distribution of Lesions.— The majority of the visible lesions are situated upon the lower legs and feet. There are a small number above the knees, both anteriorly and posteriorly, and there are two small nodules on the face and one on the back of the neck. The distribution includes the skin over the dorsal surface of the feet, the posterior surface of the ankles, the anterior aspect of the legs, the inner aspect of the calves, the knees, posteriorly the soleus muscle of the left leg, and above the popliteal space on the right leg.

The lesions consist of papules and cutaneous nodules, and also of larger and deeper subcutaneous nodules. Some of the subcutaneous nodules also involve the deeper portion of the skin. The papules and nodules situated in the skin vary from about 6 mm. to 1.5 cm. in diameter. They are usually raised for a distance of 0.5 to 1 cm. above the surface of the surrounding skin. A few are only raised about 2 mm. and these have a flattened disc-like appearance. They are sharply circumscribed, and a number of them are from pink to cherry-colored, while others are brownish.

Some of the subcutaneous nodules are larger in diameter, several measuring as much as 2.5 cm. These are situated particularly above the knees on both legs, and on the exterior surface of the left thigh. They usually move with the skin, and their margins are not sharply outlined.

During the time that the patient remained in the hospital there was little fever. The evening temperature on several occasions touched 100°.4 F. A number of the lesions were excised from time to time for histological study, and for the inoculation of animals, and numerous cultures were taken. The notes regarding these experiments will be found on page 110. Many microscopical preparations both fresh and stained were also made from the lesions. Some of the fresh preparations were examined with the dark-field microscope. No spirochaetae, acid-fast bacilli, or other visible microörganisms were detected in the unbroken lesions. For the notes of these examinations, see pages 110 to 114.

On July 16, the note regarding the case was made as follows: " There has been practically no fever. The lesions seem to be greatly improved. Many have lost their cherry-like color, and now appear either pink or grayish. Some are beginning to be brown. They also look less tense than formerly.

" The patient has received no salvarsan or mercury or iodides; nothing but tonic treatment."

On July 29 the note reads: "Patient is in greatly improved condition, and indeed one might say almost well. Almost all of the nodules and all of the miliary lesions have disappeared. Situated on the feet, and about the ankles posteriorly, a few nodules are still present. On the dorsal surface of the right foot is a tumor which is pinkish in color, and which measures about 5 mm. in diameter on the surface. It is raised for about 2 mm. above the surface of the skin. This was removed. On section it was quite moist and not dried. Another nodule, over which the skin has become dried, and resembles an onion skin, measured about 7 mm. in diameter and 5 mm. in height. It was almost the color of the normal skin. This nodule was also removed. On cut section it likewise proved to be moist in the interior."

On August 3 the patient left the hospital apparently well.

Case 19. — Extensive nodular and mulaire lesions. July 18, 1913. Male, aged twenty-nine years. Unmarried. He has one brother who is well; parents are dead. He went to work in San Bartolomé May 1 and worked there two weeks. After working at San Bartolomé he returned to Lima. He states he lived recently in a house in which there were three cases of verruga. There is an eruption upon the skin which he says appeared fifteen days ago, first on the right wrist. He had moderate fever and pains in the joints for two days before the eruption appeared.

Examination. — The mucous membranes are pale. The sclerae are clear. The left posterior cervical glands are palpable. The examination of the heart and lungs reveals nothing abnormal. The spleen is enlarged and extends 8 mm. below the costal margin. It is hard and firm. The liver is not enlarged. The blood shows no parasites. The red blood-corpuscles number 3,300,000; the leukocytes, 6,000; the hemoglobin, 45 % (Sahli). The Wassermann reaction is negative. The temperature on the day of entrance was 100°.7 F. There is an extensive eruption upon the skin.

Distribution and Character of Lesions. — One nodule on left cheek just anterior to the ear, and one on lower lip; four nodules on the neck; fourteen on the left arm and forearm; and about eighteen on the right arm and forearm; fourteen over the shoulders and neck. Three nodules on the right side of the chest, one on the left side of the chest, and one on the anterior surface of the abdomen. Seven nodules over the back. In the neighborhood of thirty or forty nodules on the legs and thighs.

No miliary lesions are present. The nodules generally measure from 2 mm. to 6 mm. in diameter. A few are larger. These measure from 1 cm. to 1.5 cm. in diameter. The lesions are raised from 2 mm. above the surface of the skin to 1 cm. All of the nodules, except a few on the feet, are of a cherry red color. They are sharply circumscribed and all are discrete. In addition to these lesions there is one of the mulaire type measuring 12

mm. in diameter at its surface, and about 8 mm. in diameter at its base. Clear serum is exuding from this lesion. There are also a number of subcutaneous nodules. One of these is situated on the elbow over the head of the radius, and is not attached to the skin. It is not sharply circumscribed and measures about 0.5 cm. in diameter. There are two similar nodules measuring about 6 mm. in diameter over both shoulders, and two more slightly larger on the flexor surface of the left forearm and one on the flexor surface of the right forearm. The palms of the hands and soles of the feet are free from lesions. There are two or three subcutaneous nodules measuring about 1 cm. in diameter on the left side of the abdominal wall, and several smaller ones to the right side of the rectus muscle. There are numerous subcutaneous nodules over both knees, and over both ankles. Some of the nodules in these localities are subcutaneous and some cutaneous. Over the cutaneous nodules the skin may appear entirely normal, or in those instances in which the nodules are more superficially situated the skin has a slight bluish red or purplish tinge. The inguinal lymphatic glands are slightly enlarged. In addition to these lesions there are three small papules (2 to 3 mm.) on the mucous membranes of the lower lip inside, one small papule 2 mm. in diameter on the glans penis, and three or four of similar size on the scrotum.

Numerous lesions of the skin were removed from time to time from this patient for study, and fresh and stained preparations were made for microscopical study. No spirochaetae, acid-fast bacilli, or other microörganisms were discovered in the preparations from the unbroken lesions. Some of the fresh preparations were examined by the dark-field microscope. For the description of these experiments and the inoculation of animals performed with material from this case, see page 114. The patient gradually improved. He had no fever during his stay in the hospital. He disappeared from the hospital towards the end of July before the lesions had entirely disappeared.

In some cases only the miliary eruption appears upon the skin, and the disease does not progress to the nodular stage. In a month the lesions may have entirely disappeared, as occurred in the following case.

Case 2. — June 20, 1913. Male. Aged thirteen years. Worked for six months in Matucana this year. None of his family are living. There is one case of verruga in the house in which he lives. The present illness began in May, 1913, with malaria and pains in the joints, followed in a few weeks with the eruption of verruga.

Physical Examination. — Mucous membranes somewhat pale. Cervical glands are not felt. Epitrochlear glands enlarged, measuring about 1×2 cm. Tonsils are moderately enlarged. Heart and lungs appear

normal. Liver is not enlarged. Spleen is just palpable at the costal margin and is hard and firm. Inguinals are not palpable. The urine is normal. The blood examination shows no parasites; the blood count shows

Red blood-corpuscles	3,000,000
Leukocytes	4,400
Hemoglobin	50 % (Sahli)

Wassermann reaction is negative.

There is an extensive miliary eruption upon the skin.

General Distribution and Description of the Lesions. — Forehead, chin, neck, particularly posteriorly, shoulders, arms, forearms, and hands (trunk entirely clear), thighs, lower legs and feet. The lesions are entirely miliary in type, measuring from pin-point to 2 mm. in diameter. Over the forehead, neck and shoulders they are grayish white in color, and are evidently receding.

Both surfaces of the right arm, forearm, and hand are involved. In the palm of the hand the majority of the lesions appear as small hemorrhagic spots deep in the skin. Over the flexor surface of the forearm the lesions are very numerous, and almost invariably discrete. They consist of grayish, brownish and pinkish papules. On the extensor surface of the hand and forearm many of the lesions are redder in color. Some are distinctly blue. On the back of the hand some of these bluish areas measure as much as 6 mm. in diameter. They are not so sharply circumscribed from the surrounding skin and some of them appear to be situated beneath it. Over the flexor surface of the arm and extensor surface there are numerous grayish white papules.

The lesions on the left arm and forearm are similar to those on the right. The soles of both feet are clear. Over the dorsal surface of both feet, anterior and posterior surface of both legs and thighs, there are very numerous papules. These are grayish, pinkish, or reddish purple in color. Although they are very numerous, and frequently closely placed, as a rule they are discrete. On pressure they do not lose their reddish color. Many of them appear as hemorrhages beneath the skin. The majority, however, are distinctly papular in character, and are firm to the touch, and give a rough feeling when the hands are passed over them. Over the internal malleoli of both ankles there are some small areas measuring about 0.5 cm. in diameter and about 1 mm. in height, which are grayish pink in color. These evidently represent confluent papules. There are also some other areas over both tibiae which are purplish in color, and which have very irregular outlines. These evidently represent hemorrhages which have occurred in the papules. Many of these areas are confluent. On pressure most, though not all, of the color is lost. The patient remained in the hospital several weeks during which time he had only an occasional rise of tempera-

ture of a degree or a degree and a half. The eruption gradually faded. No nodules appeared upon the skin or beneath it, and he was discharged apparently well.

In children the disease often runs a mild course, and the eruption is not always extensive. The child may not appear ill and its appetite may be good after the appearance of the eruption. In other cases a more copious eruption appears which is accompanied by fever. Case 7, described on page 107, and the following cases, Numbers 4 and 5, illustrate these features.

Case 4. — A child, aged fourteen months, seen at Santa Ana Hospital, Lima. The child did not appear ill and remained contented throughout the examination. The physical examination shows nothing abnormal. There are a number of lesions on the skin. The glands of the neck are barely palpable in the region of the tonsils and are not especially enlarged. The other superficial lymphatics are not enlarged. The mucous membranes appear normal. On the right cheek there is a red, slightly raised papule, measuring 2 mm. in diameter. On the right wrist is a small reddish brown papule 4 mm. in diameter. On the right shoulder is another similar papule. On the right leg there is an isolated cherry red, hemispherical elevation measuring 2 mm. across, and 1.5 mm. in height. On the inner aspect of the right thigh about 2 cm. from the inguinal furrow is a sharply circumscribed, raised papule, measuring 6 mm. in diameter, tense to the touch, and somewhat translucent. This lesion was later removed with the scissors, and after making smear preparations the remainder was fixed in 10 per cent formalin.

On the anterior surface of the right leg, 4 or 5 cm. below the knee-joint, there is a papule measuring 2 mm. in diameter, and 1 mm. in height, reddish in color, with the overlying skin dry, and slightly excoriated.

On the outer aspect of the right hip, 1 cm. anterior to the great trochanter, there is a papule measuring 3 mm. at the base, tapering cone-shaped to 1 mm. at top, and 3 mm. in height. This is pink at the base, and of a dull red to brown color at the top. It is hard to the touch, and covered by a crust. The inguinal glands are palpable, of the size of small peas. In the subcutaneous tissue on the outer aspect of the right thigh, 2 cm. above the knee, is a firm nodule, measuring 3 to 4 cm. in diameter. It is freely movable beneath the skin.

Examination of the blood is negative for parasites.

The child had practically no fever during its stay of about one month in the hospital. It took its food well and was discharged without lesions.

Case 5. — July 2, 1913. Child, aged eighteen months, of dusky brown complexion. This child is evidently ill, and whined and cried throughout the examination. The skin is hot and the child evidently has fever. The mother refuses to have its temperature taken. In the right temporal region is a rounded tumor fairly well circumscribed, with the overlying skin somewhat reddened, and measuring 1.8 cm. across. Behind the right ear is a rounded, sharply circumscribed elevation, 6 mm. across, 3 mm. in height, and of a dusky red color. On the left arm there are two papules on the flexor surface, one measuring 4 mm., the other 2 mm., of brownish red color, raised 1 to 1.5 mm. above the surface. On the forearm there is a papule measuring 3 mm. in diameter, of bright red color. Just above this lesion is a firm nodule beneath the skin, measuring 5 mm. in diameter.

The right forearm shows another small nodule beneath the skin, measuring about 4 mm. in diameter.

Distributed over the back are four papules of bright red color, slightly elevated, and measuring from 1 to 3 mm. in diameter. About 6 cm. above the umbilicus is a small red papule, measuring 3 mm. across. On the outer aspect of the thigh there is a nodule beneath the skin about 1 cm. in diameter. The right foreleg shows about nine nodules; not all are discrete. These are not sharply circumscribed. They are situated beneath the skin, which is dusky red over the central portion of the lesions, and the color fades gradually and irregularly outward.

The sole of the left foot shows an old pinkish red papule, measuring 3 mm. across, with the overlying skin smooth and tense. This case left the hospital before further observations could be made. We learned subsequently that the child had entirely recovered.

The eruption commonly does not appear upon the soles of the feet or palms of the hands. In Case 2, however, it was present upon the palms of the hands, and in the following case upon the palms and particularly upon the soles of the feet.

Case 9. — July 4, 1913. Patient lives in Callao, and comes to San Bartolomé every Friday, sleeps there and returns to Lima on Saturday. He has done this since last November with the exception of April and May, when he did not come to San Bartolomé because he was very sick. He commenced to feel ill on the 30th of March. At that time he had high fever and pain in the joints. He was ill in bed fifty days with fever, pains and chills. He became very pale and suffered with vertigo. On the 16th of May the eruption appeared. The first spot appeared on the left wrist. This lesion now appears as a dried wart covered by a blackish, brown, dried crust measuring 3.5 mm. in diameter and 2 mm. in height. This was removed, microscopical preparations made, and placed in Zenker's solution.

The remaining eruption consists of very numerous papules, usually discrete, scattered over the extensor surfaces of the hands and forearms. The palms are almost free from papules. There are four in the right palm. On the flexor surface of the wrists, however, the papules are fairly numerous, though less so than on the extensor surface. They measure from pin-point to 3 mm. in diameter and are pink in color. They are very slightly raised, not more than 0.3 to 0.5 mm. They are all dry, and none have a cherry-like appearance. Over many of them the epidermis is dry and desquamated, the lesions seem to be receding.

The same eruption appears on the soles of the feet as well as on the dorsal surface and the ankles. None above the middle of the tibia. On the feet the lesions are more hemorrhagic in character. A few are black in color from the resulting hemorrhages. Some are brownish red in the center, surrounded by pinkish areas.

As we have seen, great variation occurs in the number and size of the excrescences present on the skin in the different cases. Sometimes the eruption is very abundant, as in Cases 1, 17, and 19, and sometimes only very few larger lesions are present. In the following case but a single lesion was present during the time the case was under observation.

Case 13. — Single mulaire lesion. — July 4, 1913. A woman, aged twenty years. Lives in Matucana where she was seen. Says she has no children. The first day of May she began to feel sick. Later she had fever, became anaemic, and had pains in her joints. She had no appetite. The last of May the verruga appeared. Only one lesion is present on the skin. This consists of a tumor of the mulaire type situated over the bursa of the left elbow. It is the shape of a chestnut, and measures 18 mm. in its greatest diameter, and 12 mm. in height. It is of a dark cherry color, and firm to the touch. The patient will allow no specimens to be made, and will not allow the lesion or a portion of it to be removed. Photographs were taken. Doctor states she previously had some nodules beneath the skin which have now disappeared. She has no fever at the present time. There is very slight anaemia.

We have referred in other parts of this report to the great prevalence of malaria in the endemic centers of verruga peruviana, and to the fact that many cases of verruga peruviana have either previously had malaria or are suffering with it at the time the eruption of verruga appears. So common is a concomitant infection with verruga and malaria that some

authors have claimed that the attack of verruga predisposes to the malarial attack by lowering the resistance of the individual. In the following case which we studied there was infection both with verruga peruviana and malaria, and the febrile infection had been regarded as the Oroya fever stage of verruga.

Case 14. — *Verruga Peruviana complicated with Malaria.* — July 14, 1913. Patient lives in Matucana where he was seen. Seven years old. Born in Lima. The boy arrived here last October. Disease commenced a month ago. Had fever, much pain in the joints and back and head. Later he became anaemic. His temperature on the first day was 102°.2 F. to 103°.1 F. in the afternoon; 101°.3 F. in the morning; today it is 103°.3 F. At present there is oedema of the legs which commenced eight days ago. The eruption also commenced eight days ago. The first lesion was situated on the face. At present there are numerous miliary lesions scattered over the face, legs, forearms, and hands. These papules measure usually from 2 to 3 mm. in diameter, are raised for 1 or 2 mm. above the surface of the skin. They are not bright cherry in color, but have a grayish red appearance. Also numerous subcutaneous nodules can be felt beneath the skin, particularly at the backs of the wrists and hands, and above the knees. The nodules are quite firm to the touch. While the skin is raised over these deeper nodules it appears perfectly normal. His sister is now in Lima, and she, his mother says, also has verruga. " Her doctor thought she had malaria from the fever, but last Sunday the eruption appeared." This child is sick in bed at the present time.

A blood smear from this boy shows aestivo-autumnal malarial infection. Numerous crescents are present.

Experiments Relating to the Virus of Verruga Peruviana

Transmission to animals and cultivation of the Virus of Verruga.— Numerous inoculation experiments were made with material from the various lesions of the skin. We have already referred to the fact that we were unable to transmit the parasite of Oroya fever to animals (see page 34), and that intravenous inoculations of the blood containing the parasites *Bartonella bacilliformis* into monkeys and rabbits, and the intratesticular inoculation of rabbits produced neither local lesions, verrugas upon the skin, nor lesions elsewhere. The inoculations performed upon animals with the virus of verruga

gave positive results in these animals, and the production of definite lesions, which in the case of monkeys when the inoculation was made upon the skin very closely resembled those observed in the human cases of the disease. (Plates XXIV and XXV.)

In performing these inoculation experiments in the manner in which we did, several objects were in view: first, to determine if the disease was directly inoculable; second, to determine if a visible microörganism was present in the lesions; third, to determine if a spirochaeta was present; and fourth, to differentiate the disease from framboesia and syphilis. In this chapter, therefore, all of these points will be discussed more or less widely.

Frequently a portion of the pathological material obtained from the human verruga cases and used for making inoculations in animals was also employed in experiments in relation to the cultivation of the virus *in vitro*, and sometimes for the determination of whether the virus was of a filterable nature or whether its presence could be detected by the microscope either in stained smears or sections. In those instances in which the experiments were carried out with the same material with this diverse purpose, it is more convenient to give the notes regarding them just as they were made at the time of the experiments. The results obtained in relation to each of these problems, however, will be discussed separately after the experiments have been recounted.

Experiments with Case 1

June 20, 1913. Patient has extensive cutaneous lesions of the different types of the disease, including those of the miliary, nodular and mulaire type. After cleansing of the skin with soap and water, alcohol, ether and iodin, a number of the tumors were removed for examination as follows:

Tumor No. 1, was situated on the inner surface of the left ankle; measured about 1 cm. in diameter and 6 mm. in height above the surface of the skin, and was of the nodular type. It was sharply circumscribed from the surrounding skin, was reddish in color, and of rather firm consistence. The skin over it was perfectly smooth and rather tense.

Tumor No. 2, situated on the extensor surface of the right forearm; measured about 3 mm. in diameter and was raised above the surface of

the skin for about 1 mm. It was distinctly red in color, sharply circumscribed from the surrounding skin, and of the miliary type.

Tumor No. 3, situated on the extensor surface of the left forearm, of similar character and appearance to Tumor No. 2.

Tumor No. 4, situated over the dorsum of the left foot just below the ankle joint. Measures about 8 mm. in diameter, and projects about 4 mm. above the surface of the skin. It is dark red in color, sharply circumscribed, and the skin is smooth and rather tense over it.

Tumors Nos. 1, 2, and 3 were removed with sterile scissors, and tumor No. 4 with a sterile knife, some of the normal skin being excised with the last-mentioned tumor. Tumor No. 2 was placed immediately in corrosive alcohol solution and two-thirds of tumor No. 4 into Zenker's solution.

Cultures and Microscopical Examination. — An attempt was made to aspirate one of the nodular lesions as well as one of the larger lesions of the mulaire type, and a small amount of blood was obtained from each of these lesions which was inoculated into culture media. The excised tumors which were not placed in hardening solutions were put in sterile Petri dishes, and brought immediately to the laboratory, where they arrived about fifteen minutes after removal. They were then cut into numerous small bits with sterile forceps and scissors, the overlying skin being not disturbed and many of these bits inoculated into test tubes containing pieces of rabbit's testis, kidney and in a few instances fat. Ascitic fluid was then added to all of the tubes. To some paraffin oil was then in addition added, and to others alkaline agar. Some of the tubes were placed in sealed jars containing phosphorus under strictly anaerobic conditions. In all twenty-eight cultures were made, twelve of which were upon agar.

Many microscopical preparations were made from these cultures during the next three weeks. Some were examined with the dark-field microscope, others were stained by Giemsa's or Wright's stains, or with carbolfuchsin. No spirochaetae were detected or other microörganisms which we regarded as of any etiological significance. In several of the tubes yeast cells from the skin developed, and in several others either cocci or coarse bacilli. These cultivations, however, were only exceptionally found in the tubes. With these exceptions no definite or constant differences were observed in the appearance between the inoculated and the control uninoculated tubes. Smears for microscopical examination were also made from a number of the pieces of the tumors which remained; some of these were immediately hardened and stained, others were examined with the dark-field microscope. Rabbits Nos. 1 to 7 and Dog No. 1 (q. v.) were also inoculated with pieces of the tumors. Nothing more definite than motile granules was observed in the preparations examined with the dark-field microscope. In preparations stained by Giemsa or carbolfuchsin no spirochaetae or

other microörganisms were discovered except a few yeast cells from the skin in several of the preparations.

Inoculation of Animals

Rabbit No. 1. — Testes inoculation June 20, 1913.

Four small portions of the tumor, measuring about 1.5 to 2 mm. square, were rubbed up in 2 c.c. of saline solution in a mortar, and 0.3 c.c. of this suspension inoculated into each testis; also three drops subcutaneously beneath the right side of the scrotum. Testes measure 20 × 12 mm.

June	21,	1913.			Testes appear normal.
"	23.		Temp.	100°.0 F.	Testes appear normal at 11 A.M.
"	24.	4.30 P.M.	"	100°.2.	" red, no longer flabby.
"	25.	4.43 "	"	99°.9.	" red, no change.
"	26.	5.10 "	"	100°.5.	" red, no change.
"	30.	2.00 "	"	100°.2.	" unchanged except for minute dark spot in center of left, and in right testis small just palpable nodule on anterior dorsal surface.
July	1.	5.05 "	"	101°.	Right testis definite palpable mass; slight increase over yesterday. Left testis negative.
"	3.	9.00 A.M.	"	101°.	Definite, palpable, firm nodule in anterior portion right testis not over a few mm. Left testis is normal.
"	5.	9.08 "	"	101°.1.	Right testis, nodule firm, about as before. No marked increase. Lesion on surface of left. Visible but not palpable at site of former red spot.
"	7.	9.05 "	"	99°.8.	Right testis, definite nodule about 3–4 mm. Left testis, surface shows pale red spot 2 mm. diameter.
"	9.	10.25 "	"	100°.	Right testis, firm nodule, no marked increase. Left testis, spot beneath skin unchanged.
"	11.	10.50 "	"	99°.4.	Right testis, nodule firm, 3–4 mm. approximately. Spot on surface left testis unchanged.
"	13.	3.45 "			Right testis, firm hard nodule 3 mm., left, negative.
"	15.	10.00 "			Left, negative except spot on surface.
"	18.	11.00 "			Right testis, hard. Left, negative except minute spot.
"	21.	10.40 "			Right testis, hard. Left, negative except minute spot.
"	26.				Smaller and harder.

July 28. Killed. The right testis shows minute translucent mass in capsule. Left testis shows a firm mass in its interior. There is a bright red spot in pancreas. All the other organs appear normal. On incision of the testis this mass is found to be of firm consistence, and slightly tough. It bulges from the cut surface and has a slight yellowish tinge, less pink and transulcent than in normal tissue. It is somewhat nodular, and has a sharply circumcribed outline. Measures 6 × 5 mm. Tissue in Zenker's solution. The larger lesion photographed.

Cultures on agar were made from the lesions; these were still negative on August 1. Microscopical preparations from the lesions were negative for spirochaetae or other microörganisms.

Thirty-three other cultures were made from the lesions, small bits of them being placed in tubes of ascitic fluid, ascitic fluid + rabbit's testicle; ascitic agar + rabbit's testicle; ascitic fluid + rabbit's kidney; ascitic agar + rabbit's kidney. One-half of the liquid cultures were covered with paraffin oil. An equal number of control tubes were prepared.

Cultures were also made from the lesions upon blood-serum, egg media, and blood agar. Many microscopical preparations from the cultures were repeatedly examined during the next three weeks, some with the dark-field microscope, others were stained with Giemsa's or Wright's stains, or with carbolfuchsin. No spirochaetae or other microörganisms were found except in one tube in which cocci and another in which bacilli developed. These organisms, however, did not appear in other tubes of the same media inoculated in the same way, and were regarded as of no special significance. In the examinations made with the dark-field microscope very numerous motile granules were of course usually observed, but no definite microörganisms could be distinguished. No definite and constant changes between the inoculated tubes and many of the control ones could be detected during three weeks' observation.

Rabbit No. 2. — Testes inoculation. June 20 inoculated in similar manner to Rabbit No. 1, except no injection subcutaneously in the scrotum was made. Testes measure 21 × 12 mm.

June 21, 1913. Very slight subcutaneous swelling over left testis.

"	23.		Temp.	100°.8 F.	Testes appear normal at 11.05 A.M.
"	24.	4.24 P.M.	"	102°.8.	" " " Still soft.
"	25.	4.50 "	"	102°.5.	" " " Abscess right side of jaw.
"	26.	5.20 "	"	102°.6.	Testes appear normal. Abscess right side of jaw.
"	30.	2.00 "	"	102°.6.	Left testis, definitely palpable nodule showing increased resistance as compared with rest of testis. Barely palpable nodule in right testis.
July	1.	5.10 "	"	102°.7.	Both testes show palpable nodule deep in interior. Increase in that on left.
"	3.	9.07 A.M.	"	103°.1.	Large rounded nodule on jaw about 2 cm. diameter. Right testis shows well defined firm nodule several mm. in diameter. Central dorsal portion left testis presents a larger nodule, outline of which is becoming well defined.
"	5.	9.20 "	"	102°.4.	Abscess in jaw discharging. Right testis, nodule well defined, larger. Left much larger, surface uneven.
"	7.	9.15 "	"	100°.0.	Right testis shows distinct hard nodule; remainder of testis soft, no great increase in size. Left somewhat harder.

July 7. Killed by ether during removal of testis. The left showed larger nodule. This measured 6 mm. in diameter, bulged from cut surface, sharply limited, paler than normal surrounding tissue, pale pink, translucent, with several whiter almost opaque granules in cut surface. Right, slightly smaller nodule of same character,

surface of both slightly uneven. Smears stained with Wright's spirochaetae stain and carbolfuchsin and Gabbètt reveal no organisms. Dark-field, very minute poorly illuminated dots which appeared to have active motion. Portions of the lesions inoculated by trocar into two rabbits' testes, and others ground and suspended in saline solution and injected by syringe into two rabbits' testes. Rabbits Nos. 28 to 32 inclusive inoculated (q. v.). Cultures from the lesions were sterile after 3 weeks.

Rabbit No. 3. — Testes inoculation. Inoculated in the same way as Rabbit No. 2. Inoculation made about one hour after the removal of the tumors.

June	21, 1913.				Testes appear normal.
"	23.		Temp.	99°.1 F.	Testes appear normal at 11.10 A.M.
"	24.	4.30 P.M.	"	102°.1.	" slightly redder than formerly.
"	25.	4.45 "	"	101°.3.	" slightly redder than at first.
"	26.	5.15 "	"	101°.9.	" right slightly tenser than left.
"	30.	2.20 "	"	102°.2.	" small, uniform consistency throughout (fairly firm), except minute indefinite nodule at anterior poles.
July	1.	5.15 "	"	102°.4.	Neither testis shows any definite thickening.
"	3.	9.12 A.M.	"	101°.3.	Barely perceptible nodule middle left testis. Right testis normal.
"	5.	9.30 "	"	99°.2.	Small, barely perceptible nodule left testis, about middle.
"	7.	9.22 "	"	99°.4.	Testes: right, small hard nodule 1 mm. anteriorly. Left, larger less well defined nodule.
"	9.	10.25 "	"	101°.	Small nodule, right testis, slightly increased; firm nodule 3 or 4 mm. in left testis.
"	11.	11.00 "	"	101°.3.	Right testis, barely distinguishable unevenness. Left, rather ill-defined nodule about 3–4 mm.
"	13.	4.00 P.M.			Small definite hard nodule, anterior dorsal portion, right, and firm nodule, left testis, of larger size, 3–4 mm.

July 14. 12.15 P.M. Chloroformed. Left testis photographed after section through lesion which bulges from cut surface. It is firmer than the surrounding tissue, sharply circumscribed, and pale yellow; homogeneous and slightly opaque as compared with the translucent pink testis. Right testis also shows a lesion on side next attachment of epididymis of similar appearance to lesion in left testis not perfectly rounded. Right in corrosive alcohol 2½ hours later. Left in 10 per cent formol immediately. Remainder of lesions ground in mortar, suspended in 2.5 c.c. saline solution, and injected into Rabbits Nos. 44, 45, 46 (q. v.). Dark-field examination shows motile spermatozoa, also large pairs of rounded granules, numerous indefinite rods. Microscopical preparations stained with Wright's spirochaetal stain and carbolfuchsin negative for microörganisms. Cultures from lesion in each testicle on agar negative after fourteen days.

Rabbit No. 4.— Corneal inoculation. June 20, right cornea. Two furrows ploughed in surface. Left cornea three incisions. Inoculated with tissue from two tumors removed from Case 1 one hour previous to inoculation.

June	21, 1913.			Surface of both corneas slightly uneven.
"	23.		Temp.	99°.6 F. Surface cornea shows slightest possible unevenness at 11.08 A.M.

June	24.	4.33 P.M.	Temp.	101°.9 F.	Surface cornea smooth.
"	25.	4.50 "	"	100°.8.	" " "
"	26.	5.30 "	"	99°.8.	" " "
"	30.	2.25 "	"	101°.6.	Left cornea surface smooth. Right shows three or four shallow pits.
July	1.	3.23 "	"	101°.	Cornea smooth.
"	3.	9.20 A.M.	"	99°.7.	" surface smooth.
"	5.	9.12 "	"	99°.	Two definite shallow pits in the right cornea.
"	7.	9.25 "	"	100°.0.	Cornea, right, slight unevenness of surface around scratches.
"	9.	10.30 "	"	99°.4.	Left cornea smooth. Right, smooth.
"	11.	11.05 "			Both corneas smooth.
"	15.	10.07 "			Both corneas smooth.

July 18. Corneas smooth nearly healed. Placed with stock rabbits.

Rabbit No. 5. — Corneal inoculation. June 20 right cornea, two furrows ploughed on surface. Left cornea, three incisions. Inoculated with tissue from two verrugas removed from Case 1 about one hour previously.

June 21, 1913. Surface of both corneas slightly uneven.

"	23.		Temp.	99°.8 F.	Corneal surface smooth at 11.12 A.M.
"	24.	4.36 P.M.	"	101°.8.	Corneal surface smooth.
"	25.	4.56 "	"	102°.3.	" " "
"	26.	5.25 "	"	103°.2.	" " "
"	30.	2.45 "	"	103°.1.	Both corneas smooth.
July	1.	5.25 "	"	102°.3.	" " "
"	3.	9.30 A.M.	"	101°.9.	" " "
"	5.	9.15 "	"	99°.5.	" " "
"	7.	9.30 "	"	102°.1.	" " "
"	9.	10.30 "	"	99°.7.	" " "
"	11.	11.05 "			" " "
"	15.	10.08 "			" " "

July 18. Corneas practically healed. Placed with stock rabbits.

Rabbit No. 6. — Intraperitoneal inoculation.

June 20, 1913. Inoculated fragments of verruga nodule from Case 1 into peritoneal cavity by perforating near umbilicus with a trocar.

June	23.	Temp.	99°.5 F.	Large pustule anterior angle left eye at 11.15 A.M.	
"	24.	4.48 P.M.	Temp.	101°.8 F.	Abscess on eye discharging.
"	25.	5.03 "	"	100°.	
"	26.	5.25 "	"	101°.5.	
"	30.	2.50 "	"	102°.3.	
July	1.	5.32 "	"	102°.1.	
"	3.	9.35 A.M.	"	99°.8.	Abdomen soft.
"	5.	9.35 "	"	99°.7.	" "
"	7.	9.35 "	"	101°.3.	" "
"	9.	10.35 "	"	100°.9.	
"	11.	11.05 "	"	100°.4.	

July 15. 10.12 A.M. Temp. 100°.7. Killed. Peritoneum negative, a small amount of clear fluid. Smear shows a few degenerated cells and phagocytes. No tissue saved. Cultures made upon agar from the peritoneal fluid were still sterile on July 27.

Rabbit No. 7. — Cutaneous and subcutaneous inoculation.

June 20, 1913. A bit of verruga nodule implanted by means of trocar near umbilicus, and another near right nipple. Two scratches in skin posterior to right nipple inoculated with tissue from a verruga lesion after removal of hair.

June 21. Subcutaneous implants visible beneath skin. Scarification slightly increased.

June 23. Temp. 100°.5 F. Implants near umbilicus and near nipple barely palpable.

June 24. 4.38 P.M. Temp. 102°.0 F. Subcutaneous implants if anything increased in size. Scarifications diminishing.

June	25.	5.05 P.M.	Temp.	98°.6 F.	
"	26.	5.30 "	"	102°.2.	Implants still palpable.
"	30.	2.45 "	"	102°.8.	" " " " Implant near nipple shows definite increase in size. Cutaneous scarifications healed.
July	1.	5.30 "	"	101°.6.	Implants about as yesterday.
"	3.	9.30 A.M.	"	99°.2.	No marked increase; larger is somewhat more rounded. Both are harder.
"	5.	9.34 "	"	99°.6.	No marked increase from July 1.
"	7.	9.35 "	"	101°.5.	Subcutaneous nodules slightly diminished in size, rather harder.
"	9.	10.35 "	"	100°.9.	Implants show no definite increase.
"	11.	11.00 "			Subcutaneous implants stationary or diminishing in size.
"	15.	10.10 "	"	101°.9.	Slight diminution in size.
"	18.	11.15 "			Implants distinctly smaller. Freely movable.
"	21.	10.42 "			Implants distinctly smaller. Freely movable.

July 27, 1913. Killed. Larger implant 7 × 4 × 2 mm., smaller 5 × 6 mm. yellow gray, larger with darker gray periphery. Smear of smaller stained by Giemsa and carbolfuchsin negative for microörganisms.

Dog No. 1. Male. — Intraperitoneal and subcutaneous. June 20, 1913, inoculated subcutaneously near nipple, left side, and intraperitoneally with a bit of verruga by means of trocar. Long scratches were made on right side into which the remaining verruga tissue was thoroughly rubbed.

June 21, 1913. Implant palpable. Scarification healing.

"	23.	11.20 A.M.	Temp.	101° F.	Implant barely palpable. Scarifications represented by linear crusts. Implant marked by spot. Scarification enclosed in circle of carbolfuchsin.
"	24.	4.45 P.M.	Temp.	101°.1.	Implant shows no change; scarifications practically healed.
"	25.	5.00 "	"	100°.5.	Implant to left, hard. No increase in either. Scarification nearly healed.
"	26.		"	99°.5.	Implant diminishing in size.
"	30.	2.55 "	"	100°.9.	Implant, no increase.
July	1.	2.35 "	"	99°.2.	" " "
"	3.	9.37 A.M.	"	99°.2.	Possibly slight "
"	5.	9.37 "	"	99°.9.	Distinct increase in implant.
"	7.	9.37 "	"	100°.2.	Implant shows distinct increase in size.

July	9.	10.40 A.M.	Temp.	98°.9F.	Subcutaneous implant is increased in size. There is now a lens-shaped mass measuring about 8 mm. across and elevating the skin.
"	11.	11.10 "	"	100°.2.	Implant beneath skin 9 mm. approximately.
"	13.	4.00 P.M.			Mass of about same size, firm.
"	15.	10.15 A.M.	"	100°.2.	Implant shows further increase. Less sharply defined. Overlying skin slightly thickened.

July 16. 9.30 A.M. Killed with chloroform. A flap of skin dissected back to show nodule for photographer. It lies embedded in the subcutaneous fat and appears dark, bright red with pinkish tissues showing through. On removal it consists of a flattened lens-shaped mass measuring $1 \times 1 \times 0.25$ cm. in diameter, is firm with respect to retaining shape; on pressure thin clear fluid exudes. Grinds readily. Axillary lymph-node on left side is larger than on right and softer and more juicy. That on right firmer and pigmented. Right $8.5 \times 8 \times 1.8$ mm. Left $10.3 \times 10.2 \times 4.5$ mm. Inguinal not found on careful dissection. Minute nodes near inguinal ring. Dog had been castrated. On opening the peritoneal cavity on the lateral wall a few cm. from median line the peritoneal surface is somewhat reddened over an area 2.5×1.5 cm. The surface of this area is studded with small translucent red nodules, appearing as clear drops or vesicles. Some of these especially at periphery of area are scattered and discrete, in other places closely placed, even contiguous. They measure from mere points to 1 mm. in diameter and strongly suggest in appearance the miliary lesions often seen in man. There is a thickening on the parietal peritoneum suggesting point of puncture.

The spleen near the posterior ventral end presents an elevation on surface. On section a rounded mass is found measuring 7×7 mm. color red mottled with pale pink with a row of small nodules or follicles (0.5 to 1.5 mm.) at periphery. Edge of subcutaneous lesion placed in Zenker's solution. Lymph-nodes and spleen in 10 per cent formol and Zenker's solution. Remainder ground after $2\frac{1}{2}$ hours in salt solution and inoculated subcutaneously and intraperitoneally into Dog No. 3, testes Dog no. 2, testes Rabbit no. 47. Microscopical preparations and cultures from the lesions negative for microörganisms.

Additional Experiments, Case 1. — On June 25 the patient (Case 1) was visited, and after cleansing the skin small papules measuring 2 to 3 mm. in diameter were cut or scraped off with scissors from the face and arms. A number of microscopical smears were made from the under surface of these papules. Fresh preparations were also made from their cut surfaces, and one of the papules crushed and placed beneath a cover glass. These fresh preparations were examined with a dark-field microscope, but no definite microörganism could be detected. Spirochaetae were particularly sought for. A number of preparations stained by Giemsa, Wright, or methylene blue were studied. In some of these preparations blastomyces from the skin were fairly common and occasionally a few bacteria were seen, but nothing was observed which suggested a specific microörganism of the lesions. Fourteen cultures were made, four in ascitic fluid containing a fragment of rabbit's testis, four in ascitic fluid agar containing

rabbit's testis, and four in ascitic fluid agar containing rabbit's kidney. These media were inoculated with small pieces cut from the interior of the papules. Paraffin oil was placed on the surface of one-half of these cultures. Corresponding control tubes in equal number were prepared. Two cultures were also made upon ordinary agar.

On July 21 the note regarding these cultures reads: "Nothing definite has developed. Two of the tubes of ascitic fluid containing rabbit's testicle, covered with paraffin oil, have developed slight cloudiness as compared with the corresponding control tubes, but microscopically only fine granules can be detected in these cultures. Subcultures on agar remain sterile. Three of the tubes containing ascitic fluid agar with the fragment of tissue in the depth have remained entirely clear. One of these, and one of the tubes containing the rabbit's kidney have become contaminated with a coarse bacillus. The two agar cultures remained sterile."

July 1. The patient (Case 1) was brought to the laboratory, and the areas of skin upon which the lesions to be removed for study were situated were scrubbed with soap and water, then with alcohol and ether, and finally painted with iodin. After this disinfection the papules and larger nodules were removed with sterile forceps and scissors, and placed in sterile Petri dishes. At the same time as the removal of the papules a number of cultures were made from the serum and blood which exuded from the cut surface of the tissue after removal of the tumors. In all about ten small papules of the miliary type, and of reddish brown color, measuring about 2 to 3 mm. in diameter, were removed. Three larger nodular areas, measuring about 6, 7 and 8 mm. in diameter were also removed with scissors. One of these nodular areas, situated over the anterior surface of the right foot, and measuring about 6 mm. in diameter, and 3 or 4 mm. in height, was of a dark reddish color. Upon removal it was found in the center to contain a necrotic, cheesy, greasy-like material. Cultures and smears were made from this material.

Another tumor, measuring about 7 mm. in diameter, was situated below the internal malleolus of the same foot. It was of a dark reddish color, the surface being covered by a blood clot. A portion of this tumor had been removed by us on June 20. It was now completely removed by the scissors.

The third tumor, measuring about 8 mm. in diameter, was situated midway over the lower third of the anterior surface of the tibia. The skin over it was a little darker than the normal color. On palpation the tumor seemed a little soft in one spot, and upon removal, one area, measuring about 4 mm. in diameter, was distinctly softened and gelatinous. Cultures were made from some of this gelatinous material.

The fourth tumor, situated over the internal malleolus of the left foot, was of the mulaire type and measured about 1.5 cm. in diameter on the

surface, and had a base measuring about 6 or 8 mm. in diameter after removal. The surface was covered with a moist scab. Cultures were also made from the cut surface of this tumor after removal.

Microscopical Preparations. — Smears were made from the cut surface of the small papules, from the cut surface of the larger nodules (particularly the cheesy and gelatinous portions), from the cut surface of the tumor partially removed on June 20th, and from the cut surface of the mulaire nodule. In some of the stained preparations from the papules a few yeast cells and bacteria were encountered. Those from the cheesy and gelantinous material showed no microörganisms. In the preparations made from the tumor which had been partially removed previously, numerous yeast cells and a few bacteria were present, and in those from the mulaire lesion numerous yeast cells and very large numbers of cocci and a few bacilli were present. No spirochaetae were discovered in any of the preparations.

Cultures. — Thirty-six cultures were made with fragments of the lesions placed in ascitic fluid containing pieces of rabbit's testis or rabbit's kidney; in ascitic fluid agar containing rabbit's testis or kidney; in ascitic agar alone, on blood-serum, egg media and alkaline agar.

Some of the cultures were made by planting the miliary tumors deep into the culture media. Others were made with the gelatinous material scraped from the cut surface of one of the tumors; others from the cheesy material found in one of the tumors; and still others with small bits of tissue cut from the interior of the nodular tumor.

Other cultures were also made from the cut surface of the mulaire tumor. Half of the cultures were then covered with paraffin oil, and some placed under even more strictly anaërobic conditions in a sealed jar containing phosphorus. The cultures, together with a larger number of corresponding controls, were then placed in the incubator at thirty-seven degrees centigrade.

Cultures on agar from the mulaire lesion at its base, after removal, showed after twenty-four hours, in one tube thirty-six colonies of a white porcelain-like appearance. Microscopically the organism was a coccus and showed great variation in size (*Staphylococcus albus*). The other cultures inoculated from the mulaire lesion after three weeks in the incubator also showed this organism and some yeast cells. Some of the cultures from the other lesions after three weeks in the incubator also showed bacterial growth (usually of a coccus, or in a few instances of bacilli), but these organisms were not constantly present in the inoculated tubes comprising the different series, and from their characteristics they evidently were not concerned in the primary etiology of the lesions. In the case of culture No. 6, made in ascitic agar in which a portion of a verruga papule measuring 3 to 4 mm. was placed in the center and covered with paraffin oil, the media remained

clear for about two weeks. Subsequently along the track the tissue had taken during the inoculation of the media a distinct haziness appeared. The tube was cut and microscopical preparations and subcultures made in ascitic agar and on alkaline agar. No spirochaetae or other microörganisms could be demonstrated in the microscopical preparations made from the culture, and apparently no growth occurred in the subcultures. In culture No. 24, consisting of ascitic agar + rabbit's kidney + a bit of verruga nodule + paraffin oil, slight but distinct cloudiness also developed about the tissue and along the line of inoculation. Microscopical specimens stained with methylene blue and Giemsa's solution showed no visible microörganism, only granules. A subculture in alkaline agar gave no growth. No growth occurred in the cultures made upon blood-serum or egg media, or upon the agar tubes except those from the mulaire lesion. Of the fifteen cultures sealed and placed in the anaerobic jar with phosphorus, only the ones made from the mulaire lesion developed any growth when subcultures were made from them. Also, there were no appearances suggesting growth in the great majority of the cultures. Fresh and stained microscopical preparations, however, were made from them all, for the last time three weeks after their inoculation. No definite microörganisms were detected, though numerous granules and the remains of cells were present in all. In cultures Nos. 27 and 28, containing ascitic fluid + rabbit's testis + a fragment of verruga nodule (no paraffin oil), slight cloudiness gradually developed. Stained microscopical preparations, methylene blue and Giemsa's solution being employed, showed no microörganisms, only a large number of deeply staining granules. Subcultures on agar remained sterile. A portion of these cultures when they were one month old was inoculated into Rabbits Nos. 61 and 62 (q. v.) with a negative result.

Inoculation of Animals. — A suspension of six of the small papules, and of two of the larger tumors, measuring about 6 to 7 mm. in diameter, were ground up in 8 c.c. of saline solution. 2.5 c.c. of this suspension were then filtered through a Berkefeld filter. The remaining 5.5 c.c. of the suspension of the tumors were then injected into the testes of five rabbits (see Rabbits Nos. 8 to 12), about an equal amount of fluid being injected into each animal, and both testes being inoculated in all cases. The filtrate had been diluted with 12 c.c. of saline solution. 1 c.c. of this solution was then injected into both testes of two rabbits. From the central and inner portion of the mulaire tumor a small bit of tissue measuring about 6 mm. in diameter was removed and ground up in 2 c.c. of saline solution. One c.c. of this suspension was then injected into each testis of a rabbit.

Two other rabbits were inoculated as follows: One received an intraperitoneal inoculation with the gelatinous material remaining from the suspension used in the inoculation of Rabbits Nos. 8 to 12. Two trocar

fulls were introduced. The second was injected intraperitoneally with about 10 c.c. of the filtrate of the suspension spoken of above.

These inoculations may be summarized as follows:

Testis inoculation with suspension, of Rabbits Nos. 8, 9, 10, 11 and 12.
Testis inoculated with filtrate, Nos. 13 and 14.
Testis inoculated with verruga, mulaire type, No. 15.
Intraperitoneal inoculation with verruga suspension, No. 16.
Intraperitoneal inoculation with verruga suspension filtrate, No. 17.

Dark-Field Microscope. — Fresh preparations were made from the serum and blood from the cut surface of the miliary and nodular tumors, and from the cheesy and gelatinous material. No spirochaetae or phenomena of importance were observed.

Preservation of Material. — The following material was preserved for future histological study: (1) a slice of the mulaire lesion in Zenker's solution; (2) slices of the mulaire lesion, and one small slice of the nodular lesion previously excised, in corrosive alcohol, and one slice of the mulaire and one bit of the nodular lesion previously excised, in ten per cent formalin.

Inoculation of Animals

Rabbit No. 8. — Testes inoculation with suspension of verruga.

July 1, 1913. Inoculated both testes with 0.5 c.c. of a suspension of freshly removed small verrugas and gelatinous material from previously operated lesion, ground in salt solution.

July	3.	10.15 A.M.	Temp. 102°.6 F.	Both testes ill-defined as though ruptured. Right side of scrotum, subcutaneous oedema general. Left side, anterior part oedematous.
"	5.	8.25 "	" 99°.9.	Testes movable within scrotum. Oedema of both less tense, but more induration. Uneven on left.
"	7.	9.30 "	" 99°.9.	Scrotum normal. Left testis, small nodules scattered through substance. Right, firm nodule in anterior portion.
"	9.	10.40 "	" 100°.6.	Right, definite nodule $\frac{1}{3}$ distance from anterior end. Left testis, barely perceptible, uneven granules in substance.
"	11.	11.15 "	(not taken)	Right testis, definite nodule central portion, firm, several mm. in diameter.
"	13.	4.05 P.M.		Left, granular; right, fairly distinct firm nodule.
"	15.	10.16 A.M.		Left, slightly granular; right, fairly distinct firm nodule, rather soft, flabby.
"	18.	11.20 "		Both uneven in consistence. No definite nodule. Soft and flabby.
"	20.	10.42 "		Testes uneven consistence, small, atrophied.
"	26.	11.55 "		Testes negative, soft, uneven.

July 27. Killed. Both testes show 2 to 3 orange yellow lesions, of dry consistence, irregular in outline, 2 mm. across; each shows two lesions, otherwise atrophied. Spleen rounded, slightly enlarged. Whitish scar ventral edge liver. Emaciated, losing fur. Tissue in Zenker's solution.

Rabbit No. 9. — Testes inoculation with verruga suspension.

July 1, 1913. Inoculated both testes with 0.5 c.c. of a suspension of freshly removed small verrugas, ground in salt solution.

July	3.	10.17 A.M.	Temp.	99°.9 F.	Testes free in scrotum; tender, soft throughout.
"	5.	8.33 "	"	101°.7.	Testes free, soft, no lesion.
"	7.	10.00 "	"	100°.3.	" " " homogeneous, slight unevenness left side.
"	9.	10.45 "	"	99°.9.	Right testis normal; left normal.
"	11.	11.20 "			Both testes normal.
"	13.	4.10 P.M.			Testes slightly more tense. No definite nodules.
"	15.	10.17 A.M.			Right testis no definite lesion, tense. Left, shows a definite firm mass in central portion in addition.
"	18.	10.20 "			Right testis, indefinite nodule or induration anterior to center. Left, definite nodule apparently large.
"	21.	11.50 "			Barely perceptible nodule center left testis.
"	22.	4.00 P.M.			Chloroformed and left testis removed with only moderate aseptic precautions (alcohol). Right also now shows minute palpable nodule now that animal is tied. On section left shows pale yellowish lesion, dry, firm, bulging from surface; ½ placed in Zenker's solution. Other for inoculation of Rabbit No. 48.
"	26.	11.55 "			Left side of scrotum somewhat swollen. Right testis, a definite firm nodule in central portion.

August 3. Left scrotum, no distinct nodule. Killed. Right testis removed. This shows areas 2 × 3 mm. slightly more opaque and slightly yellower than testis, but with distinctly defined broader necrotic area. Spleen small; scar on edge of liver. Small amount of fluid and gelatinous clot in peritoneal cavity. Tissue in Zenker's solution.

Rabbit No. 10. — Testes inoculated with verruga suspension.

July 1, 1913. Inoculated both testes with 0.5 c.c. of a suspension of verruga material as in Rabbits Nos. 8 and 9.

July	3.	10.20 A.M.	Temp.	99°.4 F.	Testes movable within scrotum. Slight increase in tension. Homogeneous throughout.
"	5.	9.41 "	"	101°.1.	Testes movable, soft throughout.
"	7.	10.05 "	"	101°.1.	" " " flabby, homogeneous.
"	9.	10.45 "	"	101°.	Right scrotum slightly oedematous; left testis normal.

July	11.	11.20 A.M.	Temp.	Both testes normal.
"	13.	4.10 P.M.		Slight induration anterior pole left. Right testis negative.
"	15.	10.20 A.M.		Neither testis shows definite nodule. Left, slightly more tense. Neither flabby.
"	18.	10.25 "		Neither shows a definite nodule. Tense.
"	21.	10.50 "		Barely perceptible thickening in center of the left. Questionable in center of right.
"	22.	4.30 P.M.		Left testis excised. Testis shows a pale slightly opaque lesion 1 to 1.5 mm. across, but extending longitudinally 5 mm. or more. A portion used to inoculate Rabbit No. 49.
"	26.	11.59 A.M.		Left side of scrotum swollen. Right testis a minute firm nodule.
"	30.			Dry crust left; no distinct nodule in right.

August 3. Killed. Left side of scrotum healed. Right side barely perceptible thickening. Right testis shows one lobule more opaque and yellower than normal. Small scar at edge of liver. Spleen small. No tissue saved.

Rabbit No. 11. — Testes inoculated with verruga suspension.

July 1, 1913. Inoculated both testes with 0.5 c.c. of a suspension of verruga material as in Rabbits Nos. 8 and 9.

July	3.	10.25 A.M.	Temp. 100°.3 F.	Testes freely movable. Homogeneous, soft.
"	5.	8.50 "	" 100°.4.	Testes freely movable. Posterior end left, hard, and here slight swelling of scrotum.
"	7.	10.10 "	" 101°.4.	Right testis soft, homogeneous. Scrotum normal. Left side of scrotum swollen throughout, tense. Testis hard and fixed.
"	9.	10.50 "	" 104°.2.	Right testis normal. Left side of scrotum swollen. Testes fixed.
"	11.	11.25 "		Right testis normal. Left testis firm, swollen, with epididymis forming firm mass.
"	13.	4.15 P.M.		Right not found. Left testis fixed. Swelling less.
"	15.	10.34 A.M.		Right testis rather tense. No definite nodules. Left, fixed, indurated throughout.
"	18.			No definite nodule. Inflammation, left, subsiding, leaving posterior end firm.
"	21.			Right firm. Definite nodule in anterior portion. Left, hard posteriorly.
"	26.			Right, anterior indurated. Left, posterior portion indurated.

July 27. Killed. Right testis presents a group of lesions, $\frac{1}{2}$ used to inoculate Rabbit No. 56. Other testis shows a large yellow nodule 7 × 5 mm., on section

cheesy and soft. Smears fresh and stained, and cultures on agar negative for microörganisms. Other organs negative. Tissue in Zenker's solution.

Rabbit No. 12. — Testes inoculation with verruga suspension.

July 1, 1913. Inoculated both testes with 0.5 c.c. of a suspension of verruga material as in Rabbits Nos. 8 and 9.

July	3.	10.30 A.M.	Temp.	101°.6 F.	Oedema both sides of scrotum. Puncture of skin on right.
"	5.	8.45 "	"	100°.	Swelling of right side of scrotum; skin puckered. Testis rather hard but slips through scrotum. Left testis not found. Left scrotum normal.
"	7.	10.20 "	"	99°.	Right testis normal, homogeneous. Impossible to get left down.
"	9.	10.55 "	"	99°.6.	Slight induration at anterior and posterior end of right testis. Left normal.
"	11.	11.30 "			Definite firm nodule posterior portion right testis, and induration at anterior end. Left normal.
"	13.	4.20 P.M.			Definite firm nodule at posterior of right. Left, small, soft.
"	15.	10.30 A.M.			Firm nodule near posterior end right. Both flabby and soft.
"	18.				No definite nodule. Right, uneven consistence. Both flabby.
"	21.				No definite nodule.
"	26.				Both testes uneven. No definite nodules.

July 27. Killed. Right testis shows yellowish nodules with grayish periphery and smaller whitish gray areas within. Left, shows one minute grayish area. Other organs negative. Tissue in Zenker's solution. Fresh and stained smears with Wright and Giemsa negative for microörganisms. Cultures on agar negative.

Rabbit No. 13. — Testes inoculation with verruga filtrate. Filtrate obtained by diluting suspension used to inoculate Rabbits Nos. 8 to 12 and 15, 16, and then passing through Berkefeld filter.

July 1, 1913. Both testes inoculated with 0.5 c.c. of filtrate.

July	3.	10.10 A.M.	Temp.	100°.3 F.	Testis slightly harder on right side. No nodule in either.
"	5.	8.38 "	"	100°.	Testes, no lesion, soft, normal.
"	7.	10.25 "	"	101°.1.	" " " " "
"	9.	11.00 "	"	101°.7.	Both testes normal.
"	11.	11.30 "			" " "
"	13.	4.15 P.M.			" " " Small.
"	15.	10.40 A.M.			" " " Right slightly more tense.
"	18.				No definite nodules. Right much larger than left and more tense.
"	21.				No definite nodules. Right much larger than left and more tense.
"	26.				No definite nodules.

July 28, 1913. Killed by chloroform. The ventral edge of the liver shows whitish scar-like lesion about 2 cm. across. Other viscera show no gross lesions.

Smaller of the two testes appears normal throughout. Larger testis shows a rather translucent lobule or nodule approximately 2 mm. across, only differing from the normal in its translucency. Tissue in Zenker's solution.

Rabbit No. 14. — Testes inoculation with verruga filtrate.

July 1, 1913. Both testes inoculated with 0.5 c.c. of a filtrate obtained by diluting suspension used to inoculate Rabbits Nos. 8 to 12 and 15, 16 with 4 × volume of salt solution, and then passing through Berkefeld filter which it did quickly.

July	3.	10.07 A.M.	Temp. 99°.8 F.	Testes soft and normal.
"	5.	8.35 "	" 100°.3.	" " " "
"	7.	10.27 "	" 99°.9.	" " " flabby.
"	9.	10.55 "	" 100°.4.	Both testes normal.
"	11.	11.30 "		" " "
"	13.	4.25 P.M.		" " "
"	15.	9.42 A.M.		" " " Not flabby.
"	18.			No nodules, soft, flabby.
"	21.			" " " "
"	26.			" " " "
"	30.			Neither testis shows any nodule.

August 9. Killed. Both testes normal. Smear and cultures made. Cysticercus in omentum. No tissue saved.

Rabbit No. 15. — Testes inoculation with verruga mulaire type.

July 1, 1913. Both testes inoculated with 0.5 c.c. of a suspension of material from interior of large mulaire lesion.

July	3.	10.03 A.M.	Temp. 102°.5 F.	Testes swollen, tender, red, tense and firm.
"	5.	8.53 "	" 102°.3.	Testes slightly movable; not felt on account of swelling of scrotum which is tense, brawny and dusky purple at posterior ends of both.
"	7.	10.30 "	" 101°.	Right side of scrotum, skin normal. Testes and epididymis, dark red blotches posterior. Left side of scrotum still tense, but swelling less. Testes fixed, hard.
"	9.	11.02 "	" 102°.6.	Right testis, slightly discolored, purplish and of slightly uneven consistence. Swelling has subsided in left side of scrotum. Still some induration.
"	11.	11.35 "		Right testis, small, hard, uneven, slips readily. Left side of scrotum fairly firm nodule with opaque yellowish material beneath skin.
"	13.	4.30 P.M.		Right, homogeneous. Left, drawn up, firm cord felt.
"	15.	10.45 A.M.		Right uneven consistence. Left, palpable, firm, tender.
"	18.			Both indurated, uneven. Scrotum uneven, indurated.
"	19.			No definite nodule except abscess at tip of left scrotum.
"	26.			No definite nodule. Uneven nodules in epididymis of the left.

July 29. Killed. Testes both atrophied, and each shows several golden almost orange yellow areas definitely circumscribed but rather of more irregular form than rounded, and surface overlying is depressed as though from loss of substance. On section a quantity of whitish thick pus expressed from one side of the lesion. In addition there is a translucent nodule, not yellow, possibly the result of atrophy of the remainder of the testis. Other organs present no lesion. Microscopical preparation from the lesion in the testis shows numerous polymorphonuclear leukocytes and fair number of cocci. Culture on agar gives a rich growth of the same organism. Tissues in Zenker's solution.

Rabbit No. 16. — Intraperitoneal inoculation with verruga suspension.

July 1, 1913. Introduced greater part (2 trocars full) of gelatinous material remaining from suspension into peritoneal cavity.

July	3.	10.00 A.M.	Temp.	103°.6 F.	Thickening at point of inoculation beneath skin.
"	5.	8.58 "	"	101°.9.	Implant soft; not markedly inflamed.
"	7.	10.35 "	"	101°.9.	Implant soft; not markedly inflamed.
"	9.	11.09 "	"	102°.3.	Subcutaneous mass not increased in size.
"	11.	11.40 "			Two small accessory areas near large implant.
"	15.	10.45 "	"	101°.	Subcutaneous main implant smaller; more elevated; subsidiary larger.
"	18.	11.35 "	"	100°.8.	Implants distinctly smaller, rounder.
"	21.	11.07 "	"	102°.8.	Implants slightly smaller.
"	26.				Subcutaneous implants smaller.

July 29. Killed. The implant beneath the skin is found to consist of a sac of dark grayish caseous material mingled with whiter material. There is also a small elongated mass embedded in the muscle, and extending through the abdominal wall. Within, there is a lesion 2 mm. across, somewhat flattened, attached to the previously mentioned area by a long cord containing blood-vessels, attached to the cecum by a cord 2.5 cm. in length, and an area measuring 9 × 6 × 5 mm. This is of dull brownish color at one extremity, surrounded by a bright red zone. The remainder of the area is mottled pink, whitish and dark gray. In the cord by which this is attached is a small red area less than 1 mm. across. The larger one on section is found to have a dry surface, and is apparently for the most part necrotic. On the left lobe of the liver, near the border, is a lesion measuring 5 mm., rounded and somewhat grayish in color, but translucent. On section this is found to be somewhat cystic, and contains grayish material mingled with dark red blood. The spleen shows one minute translucent area about midway on its lateral border. The peritoneum is otherwise smooth and shows no lesions. Cultures from the cut surface of the lesions remained sterile.

Rabbit No. 17. — Intraperitoneal inoculation with verruga filtrate.

July 1, 1913. Injected about 10 c.c. of filtrate of suspension of ground-up verruga material (used to inoculate Rabbits Nos. 8 to 12 and 15, 16) into peritoneum. This suspension was diluted with four times its volume of salt solution, and it passed quickly through the Berkefeld filter, giving a clear slightly pink fluid.

July	3.	9.30 A.M.	Temp.	100°.5 F.	
"	5.	8.56 "	"	101°.5.	
"	7.	10.37 "	"	100°.8.	
"	9.	11.05 "	"	101°.6.	
"	11.	11.40 "			No observation.

July 15. 10.47 A.M. Temp. 99°.8 F. Blood smear stained by Giemsa, negative.

" 18. 10.08 " " 102°.

July 29. Killed. Peritoneum and all organs appear normal. No tissue saved.

Rabbit No. 28. — Testes inoculation 2d transfer (trocar).

July 7, 1913. Both testes inoculated by trocar with bits of lesion of Rabbit No. 2 about one hour after killing latter.

July 9. 11.35 A.M. Temp. 102°.4 F. Subcutaneous white nodule in right side of scrotum with reddening at periphery. Left testis, slight induration anterior portion.

" 11. 12.00 M. White nodule, subcutaneous, ventral aspect right side of scrotum 2 × 3 mm. No palpable nodule in interior. Left scrotum shows bluish discoloration few mm. in diameter with ill-defined border. Slight induration anterior to central portion.

" 13. 4.45 P.M. Testes rather tense. No nodule in interior, soft.

" 15. 11.23 A.M. Right not expressed. Slight swelling of posterior portion of scrotum. No definite nodule in interior.

" 18. 10.45 " Small nodules at surface of right, whitish in center, bright red at periphery. Left large, tense. No definite nodule. Point of puncture reddened and thickened.

" 19. Spot not so bright red in right testis. The left shows a large swelling in posterior portion.

" 21. Spot whitish in right testis; no nodule. Marked oedema of left scrotum.

" 26. Spot, right scrotum, extended, more diffuse. Left, harder posteriorly. No definite nodule in either.

" 29. Both practically negative. Soft.

" 30. " " " "

August 8. Right testis, palpable nodule, small. Killed. Left testis, an ill-defined area on section with dark gray markings. Right, less well defined small nodule. Testes in Zenker's solution.

Rabbit No. 29. — Testes inoculation, 2d transfer (trocar).

July 7, 1913. Both testes inoculated by trocar with bits of lesion of Rabbit No. 2 about one hour after killing latter.

July 9. 11.35 A.M. Temp. 102°.1 F. Bluish spot posterior end of right testis. Barely perceptible nodule anterior portion. Left, induration at anterior portion.

" 11. 12.05 P.M. Right no definite nodule. Left, slight induration in central portion of testis.

" 13. 4.50 " Left tense, indurated in center. Right tense, slight swelling.

July 15. 11.20 A.M. No definite nodule in either, tense.
" 18. 10.50 " " " " " " "
" 19. 3.30 P.M. " " " " " "
" 21. " " " " " "
" 26. 12.30 " " " " " "
" 29. " " " " " "
" 30. Neither testis shows any definite mass.

August 8. Killed. Left testis area of dark discoloration without reaction of surrounding tissue. Right shows area of slightly denser tissue with some discoloration. No well-defined border or bulging. Other organs normal. Tissue in Zenker's solution.

Rabbit No. 30. — Testes inoculation, 2d transfer (syringe).

July 7, 1913. Both testes inoculated with 0.5 c.c. of suspension of lesion of Rabbit No. 2 ground in salt solution, one hour after killing.

July 9. 11.40 A.M. Temp. 103°.3 F. Both testes homogeneous.
" 11. 12.05 P.M. " " normal.
" 13. 5.00 " " " "
" 15. 11.25 A.M. " " " rather tense.
" 18. 10.52 " Tense, large. No palpable nodule.
" 19. 3.20 P.M. " " " " "
" 26. 12.31 " " " " " "
" 29. " " " " "
" 30. " " " " "

August 8. Killed. Both testes show ill-defined area with some dark pigmentation. Organs normal. No culture. Tissue in Zenker's solution.

Rabbit No. 31. — Testes inoculation, 2d transfer (syringe).

July 7, 1913. Both testes inoculated with 0.5 c.c. of suspension of lesion of Rabbit No. 2 ground in salt solution one hour after removal.

July 9. 11.42 A.M. Temp. 101°.5 F. Slight thickening of skin at point of inoculation. Testis homogeneous. No testis found on left side.
" 11. 12.10 P.M. Right testis normal. Left not found.
" 15. 11.33 A.M. " " " " " "
" 18. 10.55 " " " tense. Left not found.
" 19. 3.25 P.M. " " " " " "
" 21. A.M. " " no nodules.
" 26. 12.34 P.M. " " " "
" 29. Definite nodule in right testis.
Aug. 8. " large nodule in right testis.

August 9. Killed. Right testis shows six minute elevations on surface membrane. Within its substance is a rounded nodule measuring about 5 mm. in diameter, definitely circumscribed, whitish in color. On section there appears to be no necrosis. Left is small, atrophied. Other organs appear normal. Microscopical preparations show no microörganisms. Two cultures on agar from the lesion remain sterile. Lesion ground in saline solution and portion of suspension inoculated into Rabbits Nos. 74–77 (q.v.).

Rabbit No. 32.— Intravenous inoculation, 2d transfer.

July 7, 1913. Two c.c. of suspension injected into ear vein.

July 9. 11.40 A.M. Temp. 102°.4 F.
" 11. 12.10 P.M. " 102°.5.

July 15. 11.26 A.M. Temp. 102°. F. Blood (Giemsa) negative.
" 18. 11.57 " " 101°.6.
August 8. Killed. All organs appear normal. Testes very small. No tissue saved.

Rabbit No. 44. — Testes inoculation, 2d transfer (syringe).
July 14, 1913. Both testes inoculated with 0.5 c.c. of suspension made by grinding ½ smaller lesion of Rabbit No. 3 in small amount of salt solution. Inoculation soon after killing of Rabbit No. 3.
July 15. 12.08 P.M. Both testes normal.
" 18. 12.16 " " " " Rather small, firm.
" 21. 12.02 " " " " " " "
" 26. 12.47 " " " "
" 29. Appears to be slight local swelling. No definite nodules.
Aug. 3. Left testis shows a definite nodule. Right none.
August 8. No nodule. Killed. Both testes (left larger) show rather ill-defined slightly yellowish lesions 1–3 mm. Testes in Zenker's solution. Other organs normal.

Rabbit No. 45. — Testes inoculation, 2d transfer (syringe).
July 14, 1913. Testes inoculation with 0.1 c.c. of suspension of ½ of smaller lesion of Rabbit No. 3 in salt solution in order to observe effect of small dose.
July 15. 12.08 P.M. Testes normal. Right small and soft.
" 18. 12.15 " " " " " " " "
" 21. 12.02 " " " " " " " "
" 26. 12.45 " " both normal.
" 29. Right atrophied. Left large full. No nodule.
Aug. 3. " " " " " " "
August 8. Killed. Both testes show a small yellowish nodule. Testes in Zenker's solution. Other organs normal.

Rabbit No. 46. — Testes inoculation, 2d transfer (syringe).
July 14, 1913. Both testes inoculated with 0.5 c.c. of suspension of larger lesion of Rabbit No. 3 in salt solution. This was done nearly three hours after removal.
July 15. 12.06 P.M. Soft; oedema scrotum.
" 18. 12.14 " Testes tense, normal. No oedema of scrotum.
" 21. 12 M. " " " " " " "
" 26. 12.40 P.M. " both show definite firm nodule, readily palpable.
" 29. Left testis excised, cord tied with silk, and scrotum left open. Testis shows nodule near anterior end causing surface to bulge. On section nodule whitish more opaque than testicular tissue, 3–4 mm. No yellow coloration. Microscopical preparation and culture made from lesion; and suspension of lesion inoculated into Rabbits Nos. 57, 58, 59 and 60. No tissue preserved. The microscopical preparation shows no microörganisms. The culture remained sterile.
Aug. 3. Left healed clean. Slight oedema. Right large nodule. Scrotum slightly oedematous.
Sept. 16. Scrotum somewhat thickened and possibly oedematous over central portion of testis. A small, firm mass is palpable within testis.

Sept. 19. Killed. The right testis presents a nodule of tissue of firm consistence measuring 6 × 3.5 mm., which on section bulges from cut surface and is slightly less translucent than the normal surrounding tissue and has a yellowish tint. It appears homogeneous throughout and there are no areas of necrosis. The other organs show no lesions of importance. Animal appears to have been in good health. The smaller half of the testis lesion placed in Zenker's solution. The remainder is used for inoculation of two rabbits Nos. 90 and 91 (Table 1, page 122).

Dog No. 2. — Testes inoculation.

July 16, 1913. Both testes inoculated with 0.5 c.c. of a suspension of subcutaneous nodule from Dog No. 1 ground in salt solution.

July 18. 12.20 P.M. Scrotum appears normal. Nothing over thorax.
" 21. Nothing abnormal noted.
" 26. Testis firm, movable. Slight excoriation of scrotum.
" 29. Negative.
Aug. 3. Right testis, uneven surface.

August 8. Killed by chloroform. On the right testis is a small, firm, reddish nodule. On section the anterior end shows dark discoloration with considerable reddening. This portion is apparently necrotic and more opaque than normal. Left testis shows a reddish firm nodule about 8 mm. across within its substance. It bulges slightly from the cut surface. Tissue preserved in Zenker's & Kaiserling's solutions after photograph was taken.

Dog No. 3. — Male. Subcutaneous and intraperitoneal inoculation.

July 16, 1913. Injected with 1 c.c. of same suspension as used in Dog No. 2 subcutaneously over right costal cartilages near free border. 1 c.c. of same material injected intraperitoneally to right of median line a few cm. from the inguinal line.

July 18. 12.21 P.M. Appears normal.
" 21. Nothing abnormal.
" 26. Nothing abnormal. No subcutaneous oedema.
" 29. Negative.
Aug. 3. Negative.

August 8. Killed by chloroform. Near costal margin is a small red nodule, measuring about 3 mm., embedded in the subcutaneous fat. The peritoneal surface is normal. On incision the subcutaneous lesion is found to contain a large amount of blood.

Rabbit No. 47. — Testes inoculation.

July 16, 1913. Each testis received 0.5 c.c. of suspension subcutaneous nodule from Dog No. 1.

July 18. 12.24 P.M. Testes full, normal.
" 26. 1.07 " Barely perceptible nodule in both testes; slightly larger in right.
" 29. Definite small nodule in both.
Aug. 3. Definite large " " "

August 30. Found dead. Small yellowish area found in each testis. Other organs normal. Tissue in Zenker's solution.

Rabbit No. 54. — Testes inoculation, 2d transfer (syringe).

July 22, 1913. Inoculated left testis with 0.5 c.c.; right with 0.25 c.c. of a suspension of nodule of left testis Rabbit No. 9, ground in salt solution, and used within twenty minutes.

July 26. 1.10 P.M. Neither testis shows a definite nodule. Left considerably larger and fuller.
Aug. 3. No nodules.

August 8. Killed. Right testis shows a whitish irregular area 2 or 3 mm. in diameter, and not characteristic of the verruga lesion. Left testis shows a similar opaque whitish area which is also not characteristic. Testes in Zenker's solution.

Rabbit No. 55. — Testes inoculation, 2d transfer (syringe).

July 22, 1913. Both testes inoculated with 0.5 c.c. of a suspension of portion of lesion in left testis of Rabbit No. 10.

July 26. 11.11 A.M. Testes small, normal.

Aug. 3. Definite nodule in right. Slightly smaller nodule in left testis.

August 8. Killed. Left testis shows an elongated well-defined nodule of firm consistence, pale yellow color, measures 5 × 2 cm. and extends transversely in the testis. Right testis presents a readily palpable nodule. Other organs appear normal. Tissues in Zenker's solution. In the middle of the testis on section there is a yellowish nodular mass measuring 3.5 mm. in diameter. It is well marked off from the surrounding testicular tissue and protrudes for a distance of 1 mm. above the surrounding tissue. It is firm to the touch and the surface is moist. A suspension of a portion of the lesion inoculated into Rabbit No. 70. Fresh and stained microscopical preparations reveal no microörganisms. Cultures from the lesion remain sterile.

Rabbit No. 56. — Testes inoculation, 2d transfer (syringe).

July 27, 1913. Both testes inoculated with 0.5 c.c. of suspension from testis lesion of Rabbit No. 11.

August 3. No nodules.

August 8. Killed. Left testis shows small yellowish discoloration. No characteristic lesion. Right testis shows a small yellowish area. Other organs appear normal. Tissues in Zenker's solution.

Rabbit No. 57. — Testes inoculation, 3d transfer (syringe).

July 29, 1913. Both testes inoculated with 0.5 c.c. suspension of lesion from left testis of Rabbit No. 46 ground in salt solution. This rabbit shows marked oedema of the prepuce.

August 3. Testes normal.

August 28. Killed. Organs appear normal. Both testes show a small whitish firm nodule, the largest measuring about 2 mm., the other 1.5 mm. These are ground in salt solution and a portion of the resulting suspension inoculated into four rabbits, Nos. 78 to 81. One monkey, No. 56, also inoculated with portion of same suspension. No tissue saved.

Rabbit No. 58. — Testes inoculation, 3d transfer (syringe).

July 29, 1913. Both testes inoculated with 0.5 c.c. suspension of lesion of left testis of Rabbit No. 46 ground in salt solution.

August 3. Testes normal.

Sept. 1. Found dead, considerably decomposed. Both testes placed in Zenker's solution.

Rabbit No. 59. — Testes inoculation, 3d transfer (syringe).

July 29, 1913. Both testes inoculated with 0.5 c.c. suspension of lesion of left testis of Rabbit No. 46, ground in salt solution.

August 3. No nodules.

Sept. 1. Found dead, considerably decomposed. Both testes placed in Zenker's solution.

Rabbit No. 60. — Testes inoculation, 3d transfer (syringe).

July 29, 1913. Both testes inoculated with 0.5 c.c. suspension of lesion of left testis of Rabbit No. 46, ground in salt solution.

August 3. No nodules.

Sept. 16. Rabbit thin and scrawny. Testes small, negative.

Sept. 30. Killed. Both testes small, neither shows any lesion, animal rather thin. All organs appear normal. No tissue saved.

Rabbit No. 61. — Culture inoculation into testes.

July 30, 1913. Inoculated right testis with 0.5 c.c. of cultures Nos. 27, 28. Only right testis found.

August 3. Testes small, normal.

August 28, 1913. Killed. Testes small, right somewhat injected. Neither shows any definite lesion. Lungs show numerous grayish spots varying from mere points to rounded areas 0.5 mm. in diameter, some are confluent and form larger areas of 2 or more mm. Certain of these elevate the surface of the pleura slightly. The liver shows irregular grayish areas with scar tissue near the ventral border and two cysticercus cysts found in the omentum. Tissue in Zenker's solution.

Rabbit No. 62. — Culture inoculated into testes.

July 30, 1913. Inoculated both testes with 0.5 c.c. of cultures Nos. 27 and 28 (q. v.).

August 3. Testes normal.

August 28. Killed. Both testes appear normal. Liver, spleen, lungs, and kidneys are normal. No tissue saved.

Rabbit No. 69. — Testes inoculation, 3d transfer.

August 8, 1913. Both testes inoculated with lesion from Rabbit No. 55, ground in salt solution.

September 16. Right testis small, no nodule. Left testis not found.

September 30. Killed. Testes both very small and soft; neither shows any lesions. White spots of 0.5 to 1 mm. or more on appendix. Other organs appear normal. No tissue saved.

Rabbit No. 70. — Testes inoculation, 3d transfer.

August 8, 1913. Both testes inoculated with two of the reddish nodules from omentum of Dog No. 2.

August 27. Dead. Both testes appear normal. Whitish exudate of soft consistency in the pericardium around the large vessels and the mediastinal tissue, oedematous and forming a soft clot ventral and anterior to the heart. The liver shows numerous grayish, irregular areas on its surface. Spleen is not markedly enlarged but somewhat rounded. Rabbit otherwise negative. No tissue saved.

Rabbit No. 74. — Testes inoculation, 3d transfer.

August 9, 1913. Both testes inoculated with material from lesion of Rabbit No. 31.

August 25. Found dead in afternoon. Slight post mortem decomposition. Right testis presents on surface a minute translucent red nodule. On section no lesion found in interior of either. Lungs show reddish areas which contain air throughout. Nasal mucosa shows red shiny exudate with general reddening of mucosa.

Rabbit No. 75. — Testes inoculation, 3d transfer.

August 9, 1913. Both testes inoculated with material from Rabbit No. 31.

September 16. Tip of right scrotum presents a thick crust with grayish pus underlying it. On pressure a considerable quantity of grayish pus is expressed; testis not found. Left testis presents no nodule.

September 30. Killed. Right testis for the most part destroyed, epididymis shows a small bright yellow area of necrosis. Left testis shows a single rather dense lobule, no characteristic lesion. Four lesions of cysticercus in omentum. Few old scars in the liver. Other organs normal. No tissue saved.

Rabbit No. 76. — Testes inoculation, 3d transfer.

August 9, 1913. Both testes inoculated with material from Rabbit No. 31.

September 16. Right testis negative. Left negative.

September 18. Found dead. Considerable discoloration of abdominal wall, the surface of the right testis shows a minute elevation, the interior appears normal throughout. Left testis appears normal. No tissue saved.

Rabbit No. 77. — Testes inoculation, 3d transfer.

August 9, 1913. Both testes inoculated with material from Rabbit No. 31.

September 16. Both testes negative.

September 30. Killed. Right testis shows a rather diffuse whitish coloration, but no characteristic lesion. The left testis presents a readily palpable nodule in the interior. The liver presents two nodules, one measuring 9 cm., the other 1.3 cm., of spherical form and firm consistence. On incision they are found to consist of tough sacs containing firm, dry caseous material. No acid-fast bacilli found in the stained smear. Lungs normal. Spleen of small size, shows no nodules. Other organs appear normal. Tissue in Zenker's solution. Left testis lesion ground in salt solution and used for injection of two rabbits, Nos. 98 and 99. (Table 1, page 122.) Smears from nodule in liver stained by Loeffler's methylene blue show no bacteria.

Rabbit No. 73. — Testes inoculation.

August 9, 1913. Both testes inoculated with a suspension of lesion from human lesion produced by experimental inoculation. Right 0.5 c.c., left a few drops only.

September 3. Found dead. Right testis presents a small yellowish area several mm. across. No lesion seen in left testis. Other organs normal. Tissue in Zenker's solution. Microscopical preparation and culture from the lesion in right testis show no bacteria.

Experiments with Case 16

Clinical Features. — Patient seen in Lima, at Dos de Mayo Hospital, male, aged fifteen years. Has worked in the verruga zone for several months during the present year, but has not lived with any case of verruga. Had malaria one and one-half years ago, and again six months ago. No history of fever at onset of present illness. Physical examination of the chest reveals nothing abnormal. The spleen is not palpable, and the liver not enlarged. The inguinal and epitrochlear glands are palpable. Physical examination is otherwise negative. There are no parasites observed in the blood. The red blood-corpuscles number 3,000,000; the white blood-corpuscles 4,200; hemoglobin 65 per cent (Sahli). The Wassermann reaction is positive. The urine is normal. There are a number of lesions upon the skin. Over the right elbow there is a cherry-like nodule measuring about 2.5 cm. in diameter and 1.5 cm. in height. A portion of this was previously excised for a vaccination and inoculation experiment performed

on July 9. In the left iliac region another cherry-red papule measuring 6 mm. in diameter is present. In addition there are three miliary lesions on the right forearm consisting of papules measuring from 1 to 2 mm. in diameter, and two on the left leg. There are also two subcutaneous nodules measuring from 5 to 8 mm. in diameter beneath the skin of the right forearm, and several over the tibia of each leg.

On August 3 the small papule situated on the abdomen in the left iliac region, measuring about 4 mm. in diameter, was removed with scissors, and also the mulaire lesion situated on the right elbow, after disinfection of the skin. Microscopical preparations, fresh and stained, from the cut surface of the base of the mulaire lesion and of the papule did not reveal any microörganisms. Cultures from the interior through the base of the mulaire lesion after burning of the surface, remained sterile. A portion of the mulaire lesion was preserved in Zenker's solution and about one-third of it placed in Kaiserling's solution. Another portion was used in performing antigen experiments. The following animals were inoculated with portions of the interior of the papule or mulaire lesion, or with suspensions of the same in saline solution.

Inoculation of Animals

Monkey No. 3. August 3, 1913. Inoculated on the right side of the thorax with one implant from the papule, and on the left side of the thorax with one implant from the mulaire lesion.

August 23. Over the left of the thorax a nodule the size of a split pea is palpable and distinctly visible.

August 28. A portion of the lesion on the right side of the thorax was excised and used for the inoculation of Rabbits Nos. 86 to 89, and Monkeys Nos. 8 and 9 (q. v.).

On September 30 the note reads: "Scar on right breast at present shows no redundant tissue."

Monkey No. 4.— August 3, 1913. Inoculated beneath the posterior surface of skin of the left ear, and beneath the skin of left breast with small trocar implants. The left eyebrow and eyelid, and left side of nose, and left side of abdomen were scarified and a portion of the mulaire lesion rubbed in. Soon after the inoculation the extremities of the monkey began to twitch and he died during the night. No post mortem examination was made during our absence.

Rabbit No. 37. — Testes inoculation (syringe).

July 9, 1913. Inoculated both testes with 0.5 c.c. of a suspension of interior of verruga nodule ground in salt solution. Injection about two hours after removal of tissue.

July	11.	12.25 P.M.		Right of scrotum swollen, with firm induration at posterior end. Left of scrotum normal. Testes normal.
"	12.	9.30 A.M.	Temp. 105° F.	Scrotum swollen. Testis movable.
"	15.	11.40 "	" 104°.4.	Both sides of scrotum swollen. Yellowish white areas showing through. Left side shows slough of skin 1.4 cm. in diameter with dark red surface.

July 18. 11.03 A.M. Temp. 103°.9 F. Both sides of scrotum swollen. Left, partially gangrenous with large dark crusted area.

July 19. Found dead. Nostrils clean. The right side of scrotum bluish purple, swollen, measures 3.4 cm. in length and 1.5 across. The left presents a sloughing area, measuring 2.7 in length by 1.5. There is a considerable loss of substance. The border of the opening shows whitish cheesy material. The surface of the liver shows a gelatinous, soft film of exudate, grayish color. There are irregular opaque areas showing through capsule on ventral surface, most marked near posterior border. Small amount of exudate over surface of intestine and spleen. (Tissue in Zenker's solution.) Cultures on agar from the heart, peritoneum and testes. Smear from the exudate on the surface of the liver. Smear from the liver shows large numbers of bacilli; a few long rods and many very short ones, almost like cocci, are present.

The right testis on incision showed the testicular tissue dark grayish, mingled with red. Also masses of thick creamy whitish material of puriform appearance. Small abscesses in the epididymis contain thinner whitish exudate of milky consistence. One small abscess in the cord contains white, creamy material. The left testis on section is found to be gangrenous throughout, and is surrounded by exudate forming a layer 1 mm. or more in thickness. Cultures and smears made from right testis.

Smear from the left testis shows few bacteria, short bacilli and cocci. Cultures made from left testis and from the heart and from the peritoneum on agar develop a few colonies of both cocci and colon bacilli which are not believed to bear any etiological relationship to the original verruga lesion. The mulaire tissue employed for inoculation had evidently become secondarily infected through its surface with bacteria.

Rabbit No. 38. — Testes inoculation (syringe).

July 9, 1913. Inoculated both testes with 0.5 c.c. of a suspension of the interior of a verruga nodule ground in salt solution. Injection about 4½ hours after removal of tissue.

July 11. 12.25 P.M. Rabbit's eyes closed. Discharge from nose. Difficulty in breathing. Testes both normal.

July 11. 5.00 P.M. Dead; marked rigor mortis. Austopsy at once, tissues fresh. Thin yellowish fluid runs from nose. Lungs, patchy reddening throughout. Peritoneum smooth. Smear nasal discharge shows a small bacillus and larger diplococcus. Smear from lung reveals short bacilli in leukocytes. Testes each show a slightly paler ill-defined area. A slice fixed in Zenker's solution.

Experiments with Case 7

Clinical Features. — July 4, 1913. — A child one and one-half years of age. Lives in San Bartolomé, where the patient was seen. Mother says the baby has had a very high fever, which commenced four months ago. The eruption appeared one and one-half months ago. The child is of sickly appearance. It has no fever today. There is an extensive eruption over the back of the hands, forearms and arms, legs and thighs. Mother refuses to remove clothes. States there are no lesions on the trunk and a complete examination cannot be made. The lesions consist of numerous discrete and a few confluent papules. On the legs they are

very numerous and sometimes closely placed. They are usually of the color of cherries, or a little more pink. In a few, hemorrhages have occurred, and these are of a dark blue or black color. The cherry-like tumors have a translucent appearance. They measure from about 1.5 mm. to 4 mm. in diameter, and have a height from 2 to 3 mm. There are a fair number of papules which have a pink or gray color, which do not measure more than 0.5 to 1 mm. in diameter. The eruption is very extensive and appears acute.

Note: — A blood examination shows no parasites of malaria or of Oroya fever. The child was nursing and the mother would not permit a physical examination to be made. An area of the skin upon which a number of the papules were situated was cleansed with alcohol and ether and lightly brushed with iodin solution. Four of the bright cherry-red papules were then removed with sterile forceps and scissors. These tumors were then shelled from the skin with a scalpel; three were placed in a mortar and finely ground in saline solution (0.85 per cent). The following animals were immediately inoculated with the suspension thus obtained or with bits of the fourth tumor.

Inoculation of Animals

Rabbit No. 24. — Testes inoculation by trocar.

July 6, 1913. Both testes inoculated by trocar with bits of verruga immediately after removal from child at San Bartolomé (Case 7).

July	9.	11.25 A.M. Temp. 102° F.	Distinct nodule in central portion of right testis. Also in central portion of left.
"	11.	11.50 "	The puncture point shows small crust. Surface of right testis shows a dark bluish discoloration 2 to 3 mm.; definite firm nodule in central ventral portion. Left shows minute nodule near crust of skin. Also small firm nodule in central portion of testis.
"	15.	11.12 "	Both show small yellow whitish nodule beneath skin at point of puncture. Both show small nodule in the interior.
"	18.	11.50 "	Both nodules beneath skin no larger. Both show nodules palpable in interior.
"	21.	11.22 "	Definite small nodules. No increase in either of these or in the nodules in the skin.
"	26.	12.17 P.M.	Right, small nodule within. Left, large definite nodule plainly felt. Subcutaneous nodule in scrotum not increasing.
"	30.		Right, small nodule. Left, larger definite palpable nodule.
Aug.	3.		Right, small nodule. Left, larger definite palpable nodule.

August 3. Killed. Left testis presents lesions measuring 2 to 3 mm. in diameter. Ground in mortar, and inoculated into Rabbit No. 65. Organs appear normal. Small nodules in right testis placed in Zenker's solution.

Microscopical preparations from the nodules in the testis show no spirochaetae or other visible microörganisms. Cultures remained sterile.

Rabbit No. 25. — Testes inoculation by trocar.

July 6, 1913. Both testes inoculated by trocar with bits of verruga immediately after removal from child at San Bartolomé. This rabbit's testes were very small for this mode of inoculation.

July	9.	11.25 A.M. Temp. 102°.5 F.	Testes small and apparently normal.
"	11.	11.55 "	Testes very small; minute nodule in left.
"	15.	11.10 "	No definite nodule. Testes small, unsatisfactory examination.
"	18.	11.52 "	No definite nodule.
"	21.	"	Small hard nodule at posterior tip of left testis.
"	26.	12.20 P.M.	Small hard nodule at posterior tip of left testis.
"	30.		Small. Negative.
Aug.	3.		" "

August 8. Killed. Left testis shows a nodule 2 to 3 mm. in diameter, grayish with dark marking, near periphery. Right epididymis shows a similar nodule, dark gray, measuring about 2 mm. in diameter. Liver shows two coccidial nodules. Other organs appear normal. Tissues in Zenker's solution. No culture taken. Microscopical preparations show no spirochaetae or other visible microörganisms.

Rabbit No. 26. — Testes inoculation by syringe.

July 6, 1913. Both testes inoculated with 0.5 c.c. of suspension of verrugas ground in salt solution as soon as possible after removal from child at San Bartolomé.

July	9.	11.30 A.M. Temp. 102° F.	Left testis homogeneous, right not found.
"	11.	12.00 M.	Right testis not found.
"	15.	11.15 A.M.	" " does not slip down. Left normal.
"	18.	11.54 "	Right testis does not slip down. Left normal.
"	21.		Neither testis shows any nodule.
"	26.	12.23 P.M.	Neither testis shows any nodule. Right, small, flabby.
"	30.		Left deformed. Neither shows any nodule. Right, small, flabby.
Aug.	3.		Bitten on back. Neither shows any nodule. Right, small, flabby.

August 8. Killed. Left testis appears normal throughout. Right testis atrophied, presents at both anterior and posterior poles a yellowish opaque area. Near the latter is a small translucent bulging area. Rabbit's back has been bitten, and there is a large abscess, with considerable hemorrhage beneath skin. Spleen small. Tissues in Zenker's solution. No cultures.

Rabbit No. 27. — Testes inoculation by syringe.

July 6, 1913. Both testes inoculated with 0.5 c.c. of suspension of verrugas ground in salt solution as soon as possible after removal from child at San Bartolomé. (Verruga Case 7.)

July	9.	11.30 A.M. Temp. 101° .8 F.	Both testes homogeneous except slight induration anterior end of left.

July 11.	11.55 A.M.	Testes both normal.
" 15.	11.17 "	" " " but firm and tense.
" 18.	11.55 "	" " " " " " "
" 21.	11.30 "	" " "
" 26.	12.22 P.M.	Right testis swollen posteriorly. Left negative.
" 30.		Right testis swollen posteriorly. Left apparently negative.

August 8. Killed. Both testes small. Slightly soft throughout. Slight excess of fluid in peritoneum. Organs appear normal. No cultures. No tissue saved.

Rabbit No. 65. — Testes inoculation, 2d transfer.

August 3, 1913. Inoculated both testes with suspension of material from Rabbit No. 24.

September 16. Right testis small and soft, negative. Left, small and soft, negative. Rabbit thin and fur dirty.

September 25. Found dead. Considerable post mortem decomposition. Right testis normal throughout; left also normal. A minute focus of pneumonia in lung. No tissue saved.

Experiments with Case 17

On July 10 Case 17, which showed an abundant verruga eruption (page 73 for description of the lesions present) was visited. After disinfection of the skin with soap and water, alcohol, ether, and iodin, a number of the lesions were removed. One of the subcutaneous nodules situated just superiorly and exteriorly to the right patella was first excised. Microscopical preparations were made from the interior of this nodule and also a number of cultures upon various media. (Page 111.)

Two guinea pigs (Nos. 1 and 2, page 113), and two rabbits (Nos. 42 and 43, page 112) were inoculated with a suspension of a portion of the subcutaneous nodule ground up in saline solution. Rabbits Nos. 42 and 43 were inoculated in both testes. Guinea Pig No. 1 was inoculated intraperitoneally, and Guinea Pig No. 2 intraperitoneally and into the right testis.

Two small papules, measuring 4 and 5 mm. in diameter, and 2 or 3 mm. in height, situated below the right knee, were also excised and hardened for section. A portion of the subcutaneous nodule was also hardened for section.

A third tumor, of a cherry-red color, measuring 12 mm. in diameter, and 8 mm. in height, was also excised from the toe. Much sticky serum exuded from the cut surface, which was very soft. Microscopical preparations, cultures and inoculations were also made from this nodule (page 111), as well as a portion hardened and saved for histological study.

Rabbits Nos. 39 and 40 (page 111) were inoculated into both testes, and No. 41 intraperitoneally, with a portion of the suspension of this nodule in saline solution.

Before the removal of the tumors the skin was cleansed with alcohol, ether and iodin. Disinfection over the nodule from the toe was not as thoroughly performed.

Microscopical and cultural results. — The study of the microscopical preparations from the excised papule and subcutaneous nodule in both the fresh and stained condition did not reveal any spirochaetae or other microörganisms. Wright's, Giemsa's, and methylene blue stain were employed. In the microscopical preparation made from the lesion removed from the toe, a fair number of short bacilli were present. Cultures were made from the different lesions and the following results obtained.

Two cultures were made upon alkaline agar, one from the papule and one from the subcutaneous nodule. Both of these remained sterile after two weeks. Four cultures were made from the interior of the papule in ascitic fluid covered with paraffin oil. After two weeks the fluid was still clear and microscopical examination showed no microörganisms. Subcultures on agar gave no growth. One-half cubic centimeter of each of two of these cultures was inoculated into the testes of Rabbit No. 63 (q. v.). No lesion resulted. Four cultures were made from the subcutaneous nodule in ascitic fluid agar + rabbit's testis + a fragment of the subcutaneous nodule in the center and covered with paraffin oil. Three of these cultures remained clear and showed no cloudiness after two weeks. The fourth culture showed marked cloudiness after about a week, and microscopical examination revealed in the culture a large bacillus, apparently a contamination from the air. Two cultures from the subcutaneous nodule in ascitic agar + rabbit's kidney + a fragment of the subcutaneous nodule in the center (no paraffin oil) were made. One culture remained clear after two weeks, and microscopical examination showed no visible microörganism; the other became cloudy shortly after the inoculation, and microscopical examination revealed a large pleomorphic bacillus. One culture was made from the papule removed from the toe in ascitic fluid agar + rabbit's testis + a fragment of lesion from the toe. The media became cloudy and showed a growth with production of gas after nine days. This organism present was a large bacillus and gave a heavy white growth on agar. One other culture made from the lesion of the toe in ascitic fluid + rabbit's testis + a fragment of the lesion of the toe also became cloudy and showed the same bacillus after about a week. The bacteria found in the three cultures in question obviously had nothing to do with the primary etiology of verruga.

Inoculation of Animals

Rabbit No. 39. — Testes inoculation (syringe).

July 10, 1913. Inoculated both testes with 0.5 c.c. of a suspension of interior of lesion removed from foot of Case 17.

July 15. 11.45 A.M. Testes large, flabby, normal.

July 18. 12.04 P.M. Right side of scrotum swollen, tense. Left side less swollen. Testis somewhat tense.
" 21. 11.54 A.M. Right side of scrotum greatly swollen. Left side slightly swollen. Testis negative.
" 26. 12.39 P.M. Right side of scrotum tense. Left side consists of firm mass.
" 29. Right side of scrotum — hard mass. Left side slips, but nodular throughout. Animal sickly, cold and does not eat.

July 30. Killed. Cecum shows numerous 1 mm. white lesions beneath serous membrane of appendix and ileocecal pouch. Kidneys show a few red spots 1 mm. or less. Liver, several whitish rounded nodules 2 to 3 mm. (coccidial infection). Testis, right, large with whitish, opaque masses and dry caseous exudate on surface. On section large masses of soft, dry, caseous material. Epididymis shows multiple regularly distributed areas from which thick pus exudes. Left testis shows multiple miliary abscesses. Tissues in Zenker's solution. Stained smear from appendix lesion shows a variety of bacteria in and between cells. Smears from the lesions of the left testis show a bacillus. This organism is not acid-fast and decolorizes by Gabbett's solution. A culture from the lesion in the testis shows the same organism. In glucose agar it produces gas and appears to belong to the colon group.

Rabbit No. 40. — Intravenous inoculation.

July 10, 1913. Inoculated in ear vein with 1 c.c. of suspension of same material as used in Rabbit No. 39.

July 15. 11.45 A.M. Temp. 105°.3 F. Blood shows no parasites (Giemsa stain).
" 18. 12.07 P.M. " 101°.1.

July 21. Rabbit found dead. No rigor mortis present. Body cold. Peritoneal cavity contains approximately 50 c.c. of clear watery fluid, with soft gelatinous clot. Spleen small. Kidneys injected. Two or three small grayish spots in the lung about 1 mm. across. Intestinal infection with coccidia. Smear from peritoneal fluid stained with Loeffler's methylene shows no bacteria. Culture on agar from the heart is negative. No culture taken from spleen.

Rabbit No. 41. — Intraperitoneal inoculation.

July 10, 1913. Injected 1.25 c.c. of same material used in Rabbits Nos. 39 and 40 into the peritoneal cavity.

July 15. 12 M. Temp. 102°.8 F.
" 18. 12.08 P.M. " 103°.7.
" 21. 11.52 A.M. " 102°.

August 8. Killed. Peritoneum and all organs appear normal. No tissue saved.

Rabbit No. 42. — Testes inoculation (syringe).

July 10, 1913. Inoculated both testes with 0.5 c.c. of a suspension of subcutaneous nodule from knee Case 17, ground in salt solution.

July 15. 12.02 P.M. Testes small, normal.
" 18. 12.10 " Temp. 101°.5 F. Testes small, normal.
" 21. 11.50 A.M. " 101°.6. " " "
" 26. 12.40 P.M. " " "
" 29. " " "
Aug. 3. " " "

August 8. Killed. A small nodule (yellowish) near surface of left testis. Right negative. Left testis placed in Zenker's solution.

Rabbit No. 43. — Testes inoculation (syringe).

July 10. Inoculated both testes with 0.5 c.c. of a suspension of nodule from knee of Case 17.

July 15. 12.03 P.M. Testes firm, normal.
" 18. 12.12 " Temp. 102°.6 F. Testes homogeneous.

July 19. Found dead. Body still warm, slight rigor. On opening abdominal cavity about two dozen cysticercus cysts, measuring from about 4 to 8 mm. in diameter, distributed over the omentum. The liver shows glistening, whitish nodules distributed near the ventral border of the left central lobe. On section some of the lesions are rather dry and cheesy. The lungs are of a bright red color, showing no lesions or discolorations. The fur is moist about the nostrils and mouth. Nasal mucosa clean. Tissue in Zenker's solution. Cultures from the heart show after forty-eight hours one large, white colony, evidently a contamination from the air. On longitudinal incision both testes appeared normal throughout.

Guinea Pig No. 1. — Intraperitoneal inoculation (syringe).

July 10, 1913. Inoculated 1 c.c. of suspension of nodule from knee of Case 17.

July 15. 12.04 P.M. Appears normal.
" 18. 12.18 " " "
" 21. Negative.
" 26. "

Guinea Pig No. 2. — Intraperitoneum and testes inoculation.

July 10, 1913. Inoculated intraperitoneally with 1 c.c. of suspension of knee nodule from Case 17. The right testis inoculated with 0.5 c.c. of suspension of same material.

July 15. 12.04 P.M. Appears normal. No nodules in testes.
" 18. 12.18 " " " " " " " "
" 21. 11.57 A.M. Testis inflammation, uneven induration. Animal not fat.

July 23. 10.00 A.M. Animal died during night. Peritoneum smooth, but dry and sticky. Omentum yellowish and opaque. All of left lung consolidated except apex — dark red. Smears from peritoneum, omentum, spleen and heart's blood. Those from the peritoneum and heart's blood show numerous bacilli. They are not acid-fast and decolorize easily with Gram's stain. Cultures from lower part of peritoneal cavity, omentum, and heart's blood on agar.

July 30. The culture from the peritoneal cavity and omentum shows numerous large, white colonies. This organism produces gas in glucose agar.

Peritoneal cavity washed out with 5 c.c. salt solution, and injected into a second guinea pig intraperitoneally.

Further Experiments. — On July 29 there remained on the anterior surface of the right foot a " tumor, which was pinkish in color, and which measured about 5 mm. in diameter. It was raised for about 2 mm. above the surface of the skin. This was removed. On section it was quite moist and not dried. Another nodule, on the dorsum of the foot over which the skin had become dried, and resembled an onion skin, measuring about 7 mm. in diameter and 5 mm. in height was also removed. It was almost the color of the normal skin. On cut section it likewise proved to be moist in the interior.

"The eyebrows, cheeks, nose, chin and abdomen of Monkey No. 2 were scarified, and the two tumors removed thoroughly rubbed into the scarifications within fifteen minutes after removal."

Monkey No. 2. — Cutaneous inoculation.

July 29, 1913. Visited Dos de Mayo Hospital, and excised pendulous papule and larger soft lesion from dorsum of right foot of patient. On section, interior of both were translucent, but not soft. Monkey scarified on both brows, sides of nose, inner surface of lower lip, and implants made of material of smaller lesion beneath the skin of the posterior surface of the ears. Multiple scarifications made on the belly and chin with material from the larger lesion. A portion of the large lesion and a portion of the papule placed in Zenker's solution.

August 3. Face appears smooth; brows and nose normal.

August 23. Up to present date no lesion has appeared on the face.

Jan. 21, 1914. Monkey has remained well, no eruption has appeared.

Rabbit No. 63. — Culture inoculation into testes.

July 30, 1913. Inoculated both testes with 0.5 c.c. from culture in ascitic fluid from miliary lesion Case 17.

August 3. Left slightly uneven.

August 28. Killed. Both testes appear normal. Except for scar along ventral edge of liver, organs appear normal. No tissue saved.

Experiments with Case 19

On the afternoon of July 17th about twelve papules, measuring from 3 to about 6 mm. in diameter (situated over the right arm and shoulder, and right and left leg) and one mulaire lesion measuring 11 mm. in diameter (situated over the left thigh) were removed with scissors after thoroughly cleansing the skin with soap and water, alcohol, ether and iodin. The tumors were immediately transferred to sterile Petri dishes, placed in a sterile container, and brought to the laboratory. Ten of these papules were then ground up in fifteen cubic centimeters of saline solution in a mortar, and twelve cubic centimeters of the solution filtered.

Rabbits Nos. 48 to 51 were inoculated into both testes, each with 1 c.c., and Dog No. 4 into both testes each with 1 c.c. The three cubic centimeters which remained on the filter and the fluid in the filtrate tube were then placed in a mortar and the remaining papule ground up, and three or four cubic centimeters of saline solution added. Rabbits Nos. 52 and 53 were inoculated with this suspended residue into both testes, each with 0.5 c.c., and Dog No. 5 into each testis with 0.5 c.c. and with 1 c.c. subcutaneously.

The mulaire lesion was now ground up in salt solution, and added to the remaining papular extract. A guinea pig was inoculated with 3 c.c. of this suspension intraperitoneally.

Cultures were made from one of the papules, small pieces being placed at the bottom of ascitic fluid (5 cultures) and in the middle of ascitic agar

(4 cultures) and alkaline agar (2 cultures). An equal number of similar controls was prepared. One-half of the cultures in ascitic fluid and ascitic agar were covered with paraffin oil. After twenty-four hours culture tubes and controls appeared sterile.

Cultures made from the interior of the mulaire lesion on alkaline agar showed after twenty-four hours about one hundred colonies.

A culture from the filtrate used for inoculating Rabbits Nos. 48 to 51 and Dog No. 4 on agar remained sterile after seventy-two hours.

(The inoculation of the animals was performed in from two and one-half to three and one-half hours after removal of the tumors.)

Microscopical preparations were made from the interior of the papules and from the cut surface at the base of the mulaire lesion. In the fresh and stained preparations from the papules no definite microörganism could be observed. The most striking feature was the occurrence of small, deeply-staining, blue disks in some of the preparations. These, however, had not been observed in preparations made from the lesions of other cases of verruga. Possibly they were fragments of nuclei. The reaction on the part of many of the cells to the virus of the disease seemed evident in some of these preparations, and many of them showed granules of various character, staining either deeply red or deeply blue with Giemsa's stain. No spirochaetae were detected. In the preparations from the mulaire lesion numerous cocci in clumps, pairs, or short chains were observed. An occasional bacillus was also seen. In other preparations these bacilli were not acid-fast, and decolorized easily by Gabbett's stain. The culture from the mulaire lesion on agar referred to above showed at first only white colonies. Later many of these became of a yellow color. Apparently *Staphylococcus aureus* and *albus* were present. The cultures made from the papule in special media referred to above were observed from July 17 until July 30. During this time no evidence of growth was appreciable in any of the tubes. Microscopical preparations made from the different ones, from time to time, did not reveal any visible microörganisms, but only an increasing number of deeply staining granules, probably formed by the continual disintegration of the tissue implanted. Subcultures on agar from these tubes revealed no growth. On July 30 two of the cultures made in ascitic fluid, one which had been under paraffin oil and the other without, were inoculated into the testes of rabbit No. 64 (q. v.) 0.5 c.c. of each culture being injected into each testis. No definite lesions were produced by the inoculation.

Inoculation of Animals

Rabbit No. 48. — Testes inoculation with filtrate.

July 17, 1913. Both testes inoculated with 1 c.c. of filtrate from skin nodules from Case 19.

July 21. Testes normal.

July 26. Testes normal. Rather small and firm.
" 29. " " " " " "
Aug. 3. " " " " " "

August 8. Killed. Both testes negative. Lungs show numerous discrete spots of pigmentation. One testis shows a minute translucent spot on surface. Not visible on section.

Rabbit No. 49. — Testes inoculation with filtrate.

July 17. Both testes inoculated with 1 c.c. of filtrate from skin nodules from Case 19.

July 21. Testes normal.
" 26. 12.50 P.M. " large, moderately soft, normal.
" 29. " " " " "
Aug. 3. " " " " "

Rabbit No. 50. — Testes inoculation with filtrate.

July 17. Both testes inoculated with 1 c.c. of filtrate from skin nodules from Case 19.

July 21. Normal testes.
" 26. " "
" 29. " "
Aug. 3. " "

August 8. Killed. Both testes show a minute translucent spot on surface (point of puncture). Liver an area of necrosis, and exudate on surface. Cysts and necrosis in lobes.

Rabbit No. 51. — Testes inoculation with filtrate.

July 17. Both testes inoculated with 1 c.c. of filtrate from skin nodules from Verruga 19.

July 21. Testes both normal.
" 26. " " "
" 27. " " "
Aug. 3. " " "

August 28. Killed. Both testes appear normal but of rather small size. Liver shows several whitish masses. Organs otherwise appear normal. No tissue saved.

Rabbit No. 52. — Testes inoculation, unfiltered virus.

July 17. Both testes inoculated with 0.5 c.c. of the suspension of skin nodules from Case 19, portion left upon filter resuspended. Another skin nodule was also ground and added to make up for dilution.

July 21. Both testes normal.
" 26. " " rather firm as though overlooked previously; inflamed. Definite firm nodule in left.
" 29. Definite firm nodule in right. Left, not possible to get down.

August 3. Large rounded nodule in both. Animal very emaciated. Killed by blow. The rabbit was weak and cold and extremely emaciated before being killed. No abnormality noted to account for this condition. Testes show perfectly opaque lesion yellowish in greater portion; slightly grayish pink toward periphery. Tissue in Zenker's and Kaiserling's solutions. A portion suspended and inoculated into Rabbits Nos. 66, 67, and 68 (q. v.). Microscopical preparations from the lesion of the testis show no microörganisms. Cultures on agar developed two large, white colonies of a large bacillus, perhaps a contamination from the razor used in sectioning the lesion.

Rabbit No. 53. — Testes inoculation with unfiltered virus.

July 17. Both testes inoculated with 0.5 c.c. suspension of skin lesions from Case 19 removed from filter. An additional skin lesion was added to make up for dilution.

July 21. Both testes normal.
" 26. Right no nodule. Left definite firm nodule.
" 29. Both large, firm nodules. Prepuce ulcerated and swollen.
Aug. 3. Both testes show a large firm nodule.

August 8. The left testis excised by operation. On section it shows a rounded, well-defined lesion 7 × 6 mm. across, and bulging from surface. In general it is of a pale pink color, similar to the color of the testis, but mingled with it are minute red points, and the central portion shows firm, dry, whitish, necrotic tissue. Specimen photographed. A microscopical preparation shows no bacteria. A culture on agar remained sterile. Lesion ground in saline solution and a portion of the suspension inoculated into Rabbits Nos. 71 and 72 (q. v.).

Dog No. 4. — Testes inoculation with filtrate.

July 17, 1913. Both testes inoculated with 1 c.c. filtrate of suspension of skin lesions from Case 19 within two hours after removal.

July 21.		Testes	hard,	nothing	abnormal.
" 26.	12.59 P.M.	"	"	"	"
" 29.		"	"	"	"
Aug. 3.		"	"	"	"

August 9. Killed. Testes firm and present no macroscopic lesions. All organs appear normal. No tissue saved.

Dog No. 5. — Testes and subcutaneous inoculation.

July 17, 1913. Inoculated left testis with 0.5 c.c. of suspension of skin lesions from case used in last two rabbits (Nos. 52 and 53), 1 c.c. of same material inoculated subcutaneously over right thorax.

July 21. Skin slight subcutaneous oedema. Testes hard.
" 26. Thorax shows a thickening 1.5 cm. in diameter with some diffuse swelling of the surrounding tissue.
" 29. Subcutaneous swelling more localized. Testes negative.
Aug. 3. Subcutaneous swelling smaller. Testes nothing definite.

August 9. Killed. There is a hard nodule beneath the skin of the thorax. On incision this is found to consist chiefly of tough whitish tissue, evidently of the nature of a scar, and presents nothing suggestive of a verruga lesion. The surface of both testes shows red spots about 25 to 30 in number from mere points to 3 mm. in diameter. On opening the sac these are found to be rather in the sac than in the testes proper. On close inspection these appear elevated slightly above surface and consist of flattened areas with rounded outline surrounded by delicate blood-vessels. The interior of left testis presents no lesion. Axillary lymph-nodes slightly enlarged, but greatest on side opposite to lesion. The left portion of the omentum is adherent around the spleen and shows a great number of small red rounded lesions from mere points to 2 mm. in diameter. In the region of this a small area at the edge of the spleen is of a grayish color; it is evidently necrotic, measures 2 × 4 mm. Other organs appear normal.

Rabbit No. 64. — Culture inoculation into testes.

July 30, 1913. Both testes inoculated with 0.5 c.c. culture of virus from Case 19.

August 3. Testes normal.

August 28. Killed. Both testes appear normal. All other organs appear normal. No tissue saved.

Rabbit No. 66. — Testes inoculation, 2d transfer.

August 3, 1913. Both testes inoculated with suspension of lesion from Rabbit No. 52.

August 11. Found dead. Rigor. Both testes present lesions from 1 mm. to 3 or 4 mm. opaque gray with yellow tinge, evidently necrotic in central portion. Fixed in formol. Smear stained by Wright's stain shows a small number of large bacilli with red stained centers. Cells loaded with granules, deeply stained and of varying size.

Rabbit No. 67. — Testes inoculation, 2d transfer.

August 3, 1913. Both testes inoculated with suspension of lesion from Rabbit No. 52.

August 11. Found dead. Considerable post mortem decomposition. Testis dark reddish with numerous lesions appearing as grayish opaque spots 2 to 3 mm. Testis in formol.

Rabbit No. 68. — Testes inoculation, 2d transfer.

August 3, 1913. Both testes inoculated with suspension of lesion from Rabbit No. 52.

September 16. Left side of scrotum is large and firm throughout. The testis is scarcely discernible in this mass. The right testis is somewhat swollen but presents no distinct nodules.

September 17. Found dead. Belly somewhat distended and considerable post mortem discoloration. The entire testis replaced by inflammatory tissue showing extensive necrosis. The necrotic portions are firm, yellowish white, and subdivided into alveoli. The right testis shows a large abscess at the posterior extremity containing thick yellowish puriform material. A small yellowish spot is seen in the anterior extremity. This also consists of puriform material. Post mortem decomposition rather marked. No tissue saved.

Rabbit No. 71. — Testes inoculation, 2d transfer.

August 8, 1913. Both testes inoculated with lesion of Rabbit No. 53, ground in salt solution.

August 28. Killed. One testis shows a lesion about 3 mm. in diameter with slightly yellowish tinge and opaque white in center. Nodule is of firm consistency and bulges slightly from the cut surface. Nodule in other testis is somewhat larger, about 3.5 mm. in diameter, and of similar appearance. Nodules ground in salt solution and used to inoculate four rabbits Nos. 82 to 85, and one monkey No. 67. No tissue saved.

Rabbit No. 72. — Testes inoculation, 2d transfer.

August 8, 1913. Both testes inoculated with lesion of Rabbit No. 53, ground in salt solution.

September 16. Right testis presents a minute hard nodule, the left an area 3 to 4 mm. in diameter. Scrotum normal.

September 19. Killed. Right testis is small and shows a spot on ventral surface with a small elevated area. On section there is a larger area within its substance measuring approximately 4 mm., and consisting of an aggregation of smaller lobules each of which shows a whitish interior. The left testis shows a nodule in its interior measuring 7×4.5 mm. On section it shows a considerable portion of whitish tissue evidently necrotic. Some of this is easily scraped away and is rather soft. Near the periphery the tissue is translucent with a faint yellowish tinge. Two cultures made from left testis. Half fixed in Zenker's solution, the

remainder used for inoculation of two rabbits, Numbers 92 and 93. The other organs show no lesions of importance. Rabbit was in rather poor condition, somewhat emaciated.

September 26. Two cultures from the left testis, both remained sterile after seven days.

Experiments with Case 21

Clinical data: Male, adult, observed at San Bartolomé, July 23, 1913. Patient has been living in San Bartolomé for a month or more working on the railway. There is a profuse papular eruption over both surfaces of the forearms, and backs of hands and over the legs. The papules measure from 3 to 5 mm. in diameter, are pink in color, sharply circumscribed, firm to the touch, and appear to be in a moderately active stage. The majority of them are flattened on the surface, and not dome-shaped, being raised for a distance of 1 or 2 mm. above the surface of the skin. Patient states eruption has been out about twelve days, and that he had slight fever and pain in the joints for one or two days before it appeared. Three of the papules were removed from the skin after it had been disinfected with alcohol, ether, and iodin. One was placed in Zenker's solution, one used for antigen and a third one for the inoculation of a monkey as follows:

Inoculation of Animals

Monkey No. 3. — Cutaneous inoculation.

August 1, 1913. At camp on mountain above San Bartolomé. Inoculated monkey on brows, tip of nose, inner and outer surface of lower lip and belly by scarification. A small implant made by needle in posterior surface of each ear.

August 18. Right eyelid shows linear reddened elevation. Reddish papule on left brow. Ear shows a firm mass.

August 21. Reddened elevations more distinct, now a papule on both brows.

August 23. Examination complete except inner surface of lip. Eyelid, both brows, nose and lower lip show lesions. These are reddish papules elevating the surface, the largest not over 2 or 3 mm. Those on right brow, nose and lip have been excoriated intensifying the reddening, making lesions appear more striking. Over left thorax is a lesion of the size of a split pea, similar nodule in each ear.

August 25. Papules on brows somewhat increased in size, quite red, that on nose has become somewhat rounded 2.5 to 3 mm., and is bright red. The lower lip now shows nothing definite externally.

August 28. Lesion on left eyebrow excised, and used for inoculation of four rabbits Nos. 86 to 89, and two monkeys Nos. 8 and 9 (q. v.). January 22, eyebrows normal.

Monkey No. 8. — Inoculated subcutaneously.

August 28, 1913. Inoculated on both eyebrows, tip of nose and right thorax with suspension of lesion taken from thorax of Monkey No. 3, ground in salt solution.

September 16. Cough has been present and increasing for at least a week. Considerable emaciation; animal appears pale and weak.

September 18. About 0.5 cm. beneath the nipple is a rounded nodule 5 × 4 × 2 mm. approximately.

September 20. Found dead. Small nodule about 1 cm. below right nipple situated subcutaneously. This measures about $5 \times 3.5 \times 2$ mm. and has the appearance of an abscess, being grayish white. Nose and eyebrows appear normal. Left axillary lymph-nodes are considerably enlarged and an abscess containing cheesy material the size of a mustard seed is found. The lower lobe and about one-fourth of the upper lobe of the left lung is consolidated. Near the root of the lower lobe is an abscess measuring 1.5 cm., and there are numerous small abscesses throughout this lobe. These are yellowish or grayish in color, the larger ones containing viscid puriform material. In the consolidated portion of the upper lobe are yellowish abscesses, some discrete, others confluent. There are scattered lesions throughout the other portions of the lungs. These are rather translucent and grayish with a more opaque center. Throughout the right lung are small nodules measuring from 1 to 3 mm. These are translucent at the periphery and opaque whitish gray in the center. There is one abscess containing whitish caseous material. Attached to the rib on the left thorax is an abscess about 0.8 cm. in diameter, and one of similar size and appearance in the lower aspect of the liver, left central lobe, and scattered abscesses throughout the omentum and spleen. Pericardium is smooth, shows no exudate. There is an abscess in the wall of the right ventricle and around the pulmonary artery. Tissue in Zenker's solution.

Monkey No. 9. — Cutaneous inoculation.

August 28, 1913. Inoculated on brows and tip of nose by scarification with lesion taken from eyebrow of Monkey No. 3.

September 16. Reddish rounded papules on brows measuring approximately 3 to 4 mm., and elevated 2 mm. These were well developed when seen yesterday and are apparently slightly larger today.

September 18.

Lesion A, measures $6 \times 5 \times 4$ mm., dark cherry, thin crust on surface.
" B, " $3 \times 3 \times 2.5$ mm. scraped off surface.
" C, " $2.5 \times 2.5 \times 2.5$ mm. conical.
" D, " $9 \times 3 \times 2$ mm. confluent.

September 19. Lesion " C " excised and placed in Zenker's solution. Lesion " D " excised and used for inoculation purposes (Rabbits Nos. 94 to 97, Monkeys Nos. 11 and 12, and Dog No. 6 see Tables I and II). The crust removed from lesion " A " and bits scraped off for the inoculation of Monkeys Nos. 10 and 11.

September 27. Lesion " A " presents a red granulating surface and is about the same size or has slightly increased; it appears now as before the operation. Lesion " B " shows slight change and is surmounted by a crust. There is an elevated area surmounted by a crust at site of lesion " C." Lesion " D " is now replaced by a smooth scar.

September 29. Lesion " A " somewhat increased in size, approximately spherical with red granulating surface. Lesions " B " and " C " also slightly increased.

October 17. Eyebrows appear normal.

December 3. Dead. Noticed sickly about a week ago. Autopsy immediately following death. Peristalsis observed in intestines. The lower lobe of the right lung is consolidated, also the greater part of the middle, and portions of the upper lobe. On section there are small nodules with dry grayish caseous material and abscess cavities containing slimy mucopurulent material. A smear made from the latter shows several acid-fast bacilli. The rectal mucus examined for *Balantidium coli* but none are present, although there are a few amoebae present in the mucus. There are no ulcerations. Spleen small; other organs show no lesions. Cultures on agar and bouillon. No tissue saved.

Rabbit No. 86. — Testes inoculation.

August 28, 1913. Both testes inoculated with 0.5 c.c. suspension of lesion from thorax of Monkey No. 3, ground in salt solution.

September 12. Found dead. No lesion seen in either testis, both placed in Zenker's solution. Other organs appear normal.

Rabbit No. 87. — Testes inoculation.

August 28, 1913. Both testes inoculated with 0.5 c.c. suspension of lesion from thorax of Monkey No. 3, ground in salt solution.

September 16. Both testes show nodules, left larger, not over 2 or 3 mm.

September 17. Dead. Right testis shows a small vascular elevation with a whitish center about 1 mm. in diameter. In the interior is a nodule measuring 2 mm. in diameter, of firm consistence and made up of an aggregation of small, opaque, whitish lobules with a slight yellowish tinge. The left testis shows a similar lesion just beneath the surface. In the interior is a similar nodule with less necrosis as the periphery appears somewhat translucent. The peritoneal surface is smooth. The wall of the small intestine shows a moderate number of opacities possibly coccidial lesions. The liver shows several characteristic coccidium nodules. Lungs are normal. Tissue in Zenker's solution.

Rabbit No. 88. — Testes inoculation.

August 28, 1913. Both testes inoculated with 0.5 c.c. suspension of lesion from thorax of Monkey No. 3, ground in salt solution.

September 16. Right small nodule within testis, also two firm nodules beneath skin, left fairly large nodule anterior end of testis.

September 30. Killed. Right testis shows a small lesion occupying about $\frac{1}{3}$ of the testis consisting of scattered irregular nodules without necrosis and of slightly yellowish color. Whitish opalescent nodule bulging from the surface. Left testis shows a small lesion in the interior. Other organs appear normal. Tissue in Zenker's solution.

Rabbit No. 89. — Testes inoculation.

August 28, 1913. Both testes inoculated with 0.5 c.c. suspension of lesion from eyebrow of Monkey No. 3, ground in salt solution.

September 16. Testes very small, left shows a distinct, hard nodule.

October 17. Both testes negative.

December 5. Rabbit normal.

Experiment with Case 25

August 2, 1913. The patient is an Englishman, who has been working in the mines, and has slept for three nights in the verruga district near Matucana. He was perfectly well up to June 1, 1913. Then he began to feel slightly ill. At this time he noticed a small red nodule below the right knee. This developed gradually in size. In the course of a week a second nodule developed below the right knee, and two weeks after the appearance of the first a third developed below the left knee. The patient states that he had fever at this time lasting for about ten days, and accompanied by pains in his joints and head and back. The fever left him after about ten days. The nodules have persisted up to the present time, having remained stationary for about the past two or three weeks. At present

there are three cherry red nodules, two below the right knee, measuring about 5 to 7 mm. in diameter, and one below the left knee measuring about 8 mm. in diameter. They are raised above the surrounding skin. The one on the left leg was removed with scissors, and a monkey inoculated as follows:

Monkey No. 4. — Cutaneous inoculation.

August 2, 1913. Inoculated, with portion of lesion removed from patient, right side of abdomen, face, eyebrow, and eyelid by scarification; right breast and posterior surface of right ear twice by implant.

August 3. Animal noted with eyes rolling and arms twitching, and died soon afterward.

Transmission of the Virus of Verruga to Animals

From the experiments previously recorded in this chapter in regard to the transmission of the virus to animals, and from Tables I and II, it may be seen that the monkey is the most satisfactory animal for inoculation purposes. If the skin over the eye is scarified and a small portion of a verruga nodule rubbed into the abrasions, after an incubation period usually of ten to twenty days, small papules appear which gradually enlarge and later assume the typical picture of the verruga nodules as seen in human beings. (Plates XXIV and XXV.) The nodules thus produced also have a similar histological structure to those observed in man, a fact that will be referred to again. We have transmitted this virus from animal to animal through twelve successive series of monkeys since our departure from South America over a year ago. In thirty-four monkeys typical lesions have been produced in this manner.

When the monkey is inoculated with the virus either directly from man or from another monkey, no generalized eruption occurs; as in the case when this animal is inoculated with small-pox virus only a modified form of the disease develops. It is probable that the monkey is not as susceptible to the virus of verruga or even to that of small-pox as is man. The virus of verruga also calls forth no appreciable febrile reaction in the monkey inoculated with it, and none of the monkeys died from the inoculation with this virus. The lesion in the monkey usually begins to regress four or five weeks from the time of the inoculation.

PLATE XXIV — VERRUGA INOCULATA IN THE MONKEY

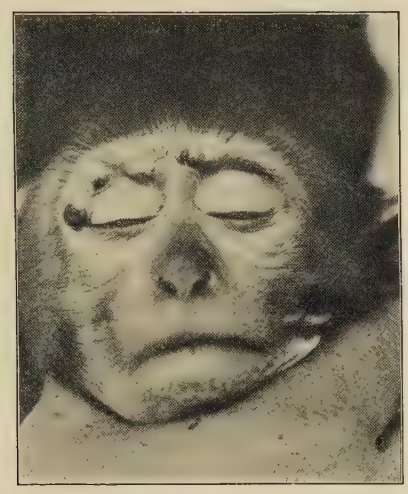


PLATE XXV.—VERRUGA PERUVIANA. Experimental lesions produced in monkeys by inoculation of human virus.

TABLE I. ANIMAL INOCULATIONS

With Material from Verruga Case 1

Animal No.	Transfer	Site of Inoculation	Result	Killed After Inocul'n
Rabbit 1	I	Testis	+	38 days
" 2	"	"	+	17 "
" 3	"	"	+	24 "
" 4	"	Cornea	−	28 "
" 5	"	"	−	28 "
" 6	"	Intraperitoneally	−	25 "
" 7	"	Cutaneously and Subcutaneously	−	37 "
Dog 1	"	Subcutaneously and Intraperitoneally	+	26 "
Rabbit 8	"	Testis	−	26 "
" 9	"	"	+	34 "
" 10	"	"	−	34 "
" 11	"	"	+	26 "
" 12	"	"	−	26 "
" 13	"	Testis with Filtrate	−	27 "
" 14	"	" " "	−	39 "
" 15	"	Testis	−	28 "
" 16	"	Intraperitoneally	−	28 "
" 17	"	" with Filtrate	−	28 "
" 28	II Rabbit 2	Testis	+	32 "
" 29	" " 2	"	−	32 "
" 30	" " 2	"	−	32 "
" 31	" " 2	"	+	33 "
" 32	" " 2	"	−	32 "
" 44	" " 3	"	−	25 "
" 45	" " 3	"	−	25 "
" 46	" " 3	"	+	67 "
Dog 2	" Dog 1	"	+	23 "
" 3	" " 1	Cutaneously	+	23 "
Rabbit 47	" " 1	Testis	+	45 "
" 54	" Rabbit 9	"	−	17 "
" 55	" " 10	"	+	17 "
" 56	" " 11	"	−	12 "
" 57	III " 46	"	+	30 "
" 58	" " 46	"	−	33 "
" 59	" " 46	"	−	33 "
" 60	" " 46	"	−	62 "
" 61	Cultures	"	−	29 "
" 62	"	"	−	29 "
" 69	III Rabbit 55	"	−	53 "
" 70	" Dog 2	"	−	19 "
" 73	II Human vaccination	"	+	25 "

Animal No.	Transfer	Site of Inoculation	Result		Killed After Inoculation
Rabbit 74	III Rabbit 31	Testis	—		16 days
" 75	" " 31	"	—	(septic)	52 "
" 76	" " 31	"	—		40 "
" 77	" " 31	"	+		52 "
" 78	IV " 57	"	—	(septic)	33 "
" 79	" " 57	"	—	"	29 "
" 80	" " 57	"	—	"	33 "
" 81	" " 57	"	—		13 "
Monkey 6	" " 57	Subcutaneously	—		..
Rabbit 90	III " 46	Testis	—		6 "
" 91	" " 46	"	—		28 "
" 98	IV " 77	"	—	(septic)	17 "
" 99	" " 77	"	—	"	17 "

With Material from Verruga Case 7

Rabbit 24	I	Testis	—	28 days
" 25	"	"	—	33 "
" 26	"	"	—	33 "
" 27	"	"	—	33 "
" 65	II Rabbit 24	"	—	53 "

With Blood from Case 8 (Oroya Fever)

Rabbit 20	Testis	—	22 days
" 21	"	—	28 "
" 22	"	—	24 "
" 23	Intravenously	—	23 "

With Blood from Case 15 (Oroya Fever)

Rabbit 33	Testis	—	28 days
" 34	"	—	2 "
" 35	"	—	28 "
" 36	Intravenously	—	28 "
Monkey 1	"	—	39 "

With Material from Verruga Case 16

Rabbit 37	I	Testis	—	10 days
" 38	"	"	—	2 "
" 73	II	"	+	25 "
Monkey 3	I	Cutaneously	+	20 "

With Material from Verruga Case 17

Rabbit 39	I	Testis	—	20 days
" 40	"	Intravenously	—	11 "
" 41	"	Intraperitoneally	—	29 "
" 42	"	Testis	—	29 "
" 43	"	"	—	9 "
Guinea Pig 1	"	Intraperitoneally	—	16 "
" " 2	"	"	—	13 "
Monkey 2	"	Cutaneously	—	..
Rabbit 63	Cultures	Testis	—	29 "

With Material from Verruga Case 19

Animal No.	Transfer	Site of Inoculation	Result		Killed After Inoculation
Rabbit 48	I	Testis with Filtrate	−		22 days
" 49	"	" " "	−		42 "
" 50	"	" " "	−		22 "
" 51	"	" " "	−		42 "
" 52	"	Testis	+		17 "
" 53	"	"	+		44 "
Dog 4	"	Testis with Filtrate	−		23 "
" 5	"	" and Subcutaneously	+		23 "
Rabbit 64	Cultures	Testis	−		29 "
" 66	II Rabbit 52	"	−	(septic)	8 "
" 67	" " 52	"	−	"	8 "
" 68	" " 52	"	−	"	41 "
" 71	" " 53	"	+		20 "
" 72	" " 53	"	+		42 "
" 82	III " 71	"	+		33 "
" 83	" " 71	"	−		33 "
" 84	" " 71	"	−		16 "
" 85	" " 71	"	−		18 "
" 92	" " 72	"	−		28 "
" 93	" " 72	"	−		11 "
" 100	IV " 82	"	+		17 "

With Material from Verruga Case 21

Animal No.	Transfer	Site of Inoculation	Result	Lesions Present After Inocul'n
Monkey 3	I	Cutaneously	+	27 days
Rabbit 86	II Monkey	Testis	−	
" 87	" "	"	+	20 "
" 88	" "	"	+	19 "
" 89	" "	"	−	
Monkey 8	" "	Cutaneously and Subcutaneously	−	
" 9	" "	" " "	+	18 to 53 days
Rabbit 94	III "	Testis	+	28 days
" 95	" "	"	+	28 "
" 96	" "	"	+	28 "
" 97	" "	"	+	28 "
Monkey 10	" "	Cutaneously and Subcutaneously	+	8 to 28 days
" 11	" "	" " "	+	10 " 28 "
" 12	" "	" " "	+	8 " 28 "
Dog 6	" "	Subcutaneously	+	11 " 25 "
Monkey 13	IV " Filtrate	Cutaneously	−	
" 14	" " "	"	−	
" 15	" " "	"	−	
" 16	" " Virus	"	+	
" 17	" " "	"	+	

With Material from Verruga Case 24

Animal No.	Transfer	Site of Inoculation	Result	Died After Inocul'n
Monkey 4	I	Subcutaneously and Cutaneously	−	2 days

TABLE II. INOCULATION OF MONKEYS WITH VIRUS OF VERRUGA PERUVIANA

Transfer No.	How Inoculated	Animal No.	Date of Inoculation	Date of Appearance of Lesions	Remarks
1	With blood from Case 15	1	July 27, 1913	....	Negative. Dead, Aug. 15, 1913.
1	With lesions from Case 17	2	July 29, 1913	....	Negative.
6	With lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	Feb. 16, 1914	Numerous papules. Well, Sept. 29, 1914.
1	With lesions from Cases 16 and 21	3	Aug. 1, 1913	Aug. 18, 1913	Eruption abundant. Lesion used to inoculate Monkeys 8 and 9, and Rabbits 86–89.
6	With lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	....	Negative. (Immune.)
1	With lesion from Case 24	4	Aug. 2, 1913		
1	With lesion from Case 16	..	Aug. 3, 1913	....	Dead, Aug. 3, 1913.
4	Subcutaneously with suspension of lesions in testes of Rabbit 57	6	Aug. 28, 1913	....	Negative.
6	With lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	Feb. 2, 1914	Numerous pale red papules.
3	Subcutaneously with suspension of lesion from testis of Rabbit 71	7	Aug. 28, 1913	....	Negative.
6	By scarification with lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	Feb. 2, 1914	Red rounded papules and large nodular lesions. Eruption moderate.
2	Subcutaneously with suspension of lesion from Monkey 3	8	Aug. 28, 1913	....	Negative. Dead, Sept. 20, 1913. Tuberculosis.

2	Cutaneously with lesion from Monkey 3	9	Aug. 28, 1913	Sept. 16, 1913	Eruption abundant. Lesion " A " used to inoculate Monkeys 10 and 11. Lesion " D " used to inoculate Rabbits 94–97, Monkeys 11 and 12, and Dog 6. Dead, Dec. 3, 1913. Tuberculosis.
3	By scarification and implants from lesion " A " from Monkey 9	10	Sept. 19, 1913	Sept. 27, 1913	Eruption abundant.
6	By scarification with lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	....	Negative. (Immune.)
3	By scarification and stab with lesions " A " and " D " from Monkey 9	11	Sept. 19, 1913	Sept. 29, 1913	Eruption abundant.
6	By scarification with lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	....	Negative. (Immune.)
3	By scarification and stab with lesion " D " from Monkey 9	12	Sept. 19, 1913	Sept. 27, 1913	Eruption moderate.
6	By scarification with lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	....	Negative. (Immune.)
4	By scarification and stabs with filtrate from lesions of Monkeys 10 and 11	13	Oct. 8, 1913	....	Negative. Dead, Nov. 13, 1913. Tuberculosis.
4	By scarification and stabs with filtrate from lesions of Monkeys 10 and 11	14	Oct. 8, 1913	....	Negative.

TABLE II. — Continued

Transfer No.	How Inoculated	Animal No.	Date of Inoculation	Date of Appearance of Lesions	Remarks
5	By scarification with tissue from lesion of Monkey 16	..	Reinoculated, Dec. 11, 1913	Jan. 13, 1914	Eruption slight. Nodules used to inoculate Monkey 29. Dead, Jan. 16, 1914.
4	Subcutaneously and by scarification and stabs with filtrate from lesions of Monkeys 10 and 11	15	Oct. 8, 1913	....	Negative.
5	By scarification with tissue from Monkey 16	..	Reinoculated, Dec. 11, 1913	Jan. 13, 1914	Nodules used to inoculate Cats 1 and 2, Monkeys 27 and 28, and for glycerinized virus. Eruption abundant. Nodules on eyebrow and nose used to inoculate all previously inoculated animals in stock except last two inoculated, viz., Monkeys 6, 7, 10, 11, 12, 16, 20, 22, 23, and 26.
4	By stabs with unfiltered material from lesions of Monkeys 10 and 11	16	Oct. 8, 1913	Oct. 17, 1913	Nodules on eyebrows used for inoculation of Monkeys 18 and 19 and for cultures. Later 2 large papules excised and used for filtration and also for inoculation by scarification of Monkeys 14 and 15.
6	By scarification with lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	....	Negative. (Immune.)
4	By stabs with unfiltered material from lesions of Monkeys 10 and 11	17	Oct. 8, 1913	Nov. 10, 1913, lesions present	Papules from eyebrow used for inoculation of Monkey 20. Dead, Nov. 11, 1913.

5	By stabs with tissue from lesions of Monkey 16	18	Nov. 4, 1913	Nov. 25, 1913	Two nodules excised from eyebrow for inoculation of Monkeys 24 and 25. Eruption abundant. Dead, Jan. 12, 1914.
5	By stabs with tissue from lesions of Monkey 16	19	Nov. 4, 1913	Dec. 7, 1913, eruption present	Eruption moderate. Dead, Dec. 8, 1913.
5	By scarification with tissue from dead Monkey 17	20	Nov. 11, 1913	Dec. 9, 1913	Eruption moderate.
6	With lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	....	Negative. (Immune.)
	By scarification with Culture No. 1 from lesion of Monkey 16	21	Nov. 19, 1913	....	Negative. Dead, Jan. 19, 1914.
	By scarification with Culture No. 2 from lesion of Monkey 16	22	Nov. 19, 1913	Dec. 9, 1913	Small reddish papules arranged in a row on both brows.
6	By scarification with lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	Feb. 9, 1914	Eruption very slight surmounted by crusts.
	By scarification with Cultures 16 and 17 from lesion of Monkey 16	23	Nov. 19, 1913	....	Negative.
6	With lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	Jan. 28, 1914	Eruption slight. Lesions used for inoculation of eggs for cultivation. Dead, Feb. 18, 1914.
6	By scarification with tissue from lesion of Monkey 18	24	Nov. 25, 1913	Dec. 9, 1913	Eruption moderate. Dead, Jan. 12, 1914.
6	By scarification with tissue from lesion of Monkey 18	25	Nov. 25, 1913	Dec. 9, 1913	Eruption abundant. Dead, Jan. 12, 1914.

TABLE II. — Continued

Transfer No.	How Inoculated	Animal No.	Date of Inoculation	Date of Appearance of Lesions	Remarks
5	Subcutaneously with filtrate from lesion of Monkey 16	26	Dec. 11, 1913	....	Negative.
6	By scarification with lesion from Monkey 15	..	Reinoculated, Jan. 22, 1914	Feb. 2, 1914	Eruption slight. Dead, Mar. 10, 1914.
6	By scarification with tissue from lesion of Monkey 15	27	Jan. 13, 1914	....	Negative. Dead, Jan. 19, 1914.
6	By scarification and implant with tissue from lesion of Monkey 15	28	Jan. 13, 1914	Jan. 28, 1914	Eruption slight. Eyebrows negative at death. Dead, Mar. 29, 1914.
6	By scarification and implant with tissue from lesion of Monkey 14	29	Jan. 13, 1914	Jan. 28, 1914	Lesions used for cultivation of virus in eggs, and for inoculation of Monkeys 30 and 33. Eruption slight. Dead, Mar. 10, 1914.
	By scarification with glycerinized virus from lesions of Monkey 15	30	Jan. 23, 1914	....	Negative.
7	By scarification and implant with lesion from Monkey 29	..	Reinoculated, Feb. 28, 1914	Mar. 28, 1914	Lesions very small.
	By scarification with glycerinized virus from lesion of Monkey 15	31	Jan. 23, 1914	....	Negative. Dead, Feb. 28, 1914.

	By scarification with dried lesions from Monkey 15	32	Jan. 23, 1914	....	Negative.
9	By scarification and implant with lesion from Monkey 35	..	Reinoculated, April 25, 1914	May 20, 1914	Eruption moderate. Lesion used for inoculation of Monkeys 27 and 36.
7	By scarification and implant with lesion from Monkey 29	33	Feb. 28, 1914	Mar. 28, 1914	Eruption abundant. Nodules used for inoculation of Monkeys 34 and 35.
8	By scarification and implant with lesion from Monkey 33	34	April 3, 1914	April 21, 1914	Eruption slight. Dead, May 15, 1914.
8	By scarification and implant with lesion from Monkey 33	35	April 3, 1914	April 21, 1914	Eruption abundant. Nodule used for inoculation of Monkey 32.
9	By scarification and implant with lesion from Monkey 32	36	May 22, 1914	June 15, 1914, lesions present	Eruption abundant. Lesions used for Monkeys 38 and 39. Dead, June 23, 1914.
9	By scarification and implant with lesion from Monkey 32	37	May 22, 1914	June 15, 1914, lesions present	Eruption slight. Dead, June 16, 1914.
10	By scarification and implant with lesion from Monkey 36	38	June 16, 1914	July 12, 1914, lesions present	Eruption slight. Lesion used to inoculate Monkeys 40 and 41. Dead, July 26, 1914.
10	By scarification and implant with lesion from Monkey 36	39	June 16, 1914	July 15, 1914, lesions present	Eruption moderate. Dead, July 17, 1914.

TABLE II. — Continued

Transfer No.	How Inoculated	Animal No.	Date of Inoculation	Date of Appearance of Lesions	Remarks
11	By scarification with lesion from Monkey 38	40	July 14, 1914	Aug., 1914, lesions present	Eruption slight. Well in Oct., 1914.
11	By scarification and implant with lesion from Monkey 38	41	July 14, 1914	Aug. 19, 1914	Eruption slight. Lesion used to inoculate Monkeys 42 and 43, and Cats 3 and 4. Well in Oct., 1914.
12	By scarification and implant with lesion from Monkey 41	42	Aug. 19, 1914		Slight eruption.
12	By scarification and implant with lesion from Monkey 41	43	Aug. 19, 1914		Negative.
13	By scarification and implant with lesion from Cat 3	44	Oct. 21, 1914		Negative.
14	By scarification and implant of material from Monkey 44	45	Nov. 11, 1914		Negative.

From the experiments recorded in this section, and from Table I, it may also be seen that the virus may be sometimes transmitted to rabbits and to dogs. In the dog the subcutaneous inoculation occasionally produces a lesion which is much more vascular than the one produced in the rabbit's testis. Upon intratesticular inoculation of the virus into rabbits or dogs, a more or less characteristic lesion is sometimes produced after an incubation period varying usually from ten to twenty-two days. (Plate XXVI.) The lesion usually measures several millimeters in diameter, and is sharply circumscribed from the surrounding tissue. In the rabbit's testis it suggests in its appearance not a lesion which has resulted from an acute inflammatory process, but rather one which has arisen from a rather low grade of inflammation. By repeated inoculations of this character, however, the virus becomes gradually attenuated, and finally inoculations of this nature fail. In only a certain percentage of the inoculated rabbits does a lesion develop, as may be seen from Table I. This fact seems to show that rabbits are not very susceptible to the virus.

In the earlier literature upon the subject of verruga peruviana and Oroya fever, it has been repeatedly stated that the lower animals were susceptible to the disease, and that the infection had been observed in dogs, cats, mules, and poultry. Scheube and Manson refer to these statements. Manson points out in this connection that if the lower animals were susceptible to the disease, it is curious that Carrion chose to experiment upon himself. Scheube calls attention to the fact that animals have also been reported to suffer from Oroya fever. Barton claimed the production of a verruga-like eruption upon the skin of dogs following the inoculation of an organism said to have been cultivated from cases of Carrion's fever, and afterwards shown to be a paracolon bacillus. As a matter of fact there was no demonstration that the virus of verruga could be transmitted to animals until experiments with monkeys were undertaken. Jadassohn and Seiffert [1]

[1] Jadassohn and Seiffert: Ztschr. f. Hyg. u. Infectionskrankh., Leipz., 1910, lxvi, 247.

in one case of verruga peruviana, and Mayer, Rocha-Lima, and Werner [1] in another case, both of which were studied in Europe, also succeeded in transmitting the virus to monkeys through several generations. Cole [2] showed from a study of Jadassohn's case, and the material from the animal lesions, that the histological structure of the nodules produced in the monkeys was also similar to that of the human lesions. In Lima more recently Dr. Ribeyro also transmitted the virus of verruga to a monkey in which we observed the lesions develop. So far as we know up to the time of writing no publication of this experiment has yet been made. [3] These experiments and our own upon monkeys, dogs, and rabbits, already described, are the only ones that have demonstrated that the virus of verruga is transmissible to the lower animals. We did not observe any cases of natural infection of the disease in dogs, cats, donkeys, or poultry, and do not believe the disease occurs naturally in these animals. In fact the monkey is the only really satisfactory animal for inoculation purposes in this disease. In the transmission of the virus from man to animals it is noticeable that in the monkey, and as has been mentioned particularly in the intratesticular inoculation of the rabbit, the inoculation often fails, and no lesion results. This may be seen from Tables I and II, and is particularly striking in the experiments performed with Cases 1, 7, 17, 19, and 21. It would appear that the virus, even in man, is only transmissible during a certain stage of the disease, and that unless it is obtained from the lesion of the human case during that stage, it is either innocuous for animals or its virulence is greatly reduced. Also, the susceptibility of the animal to the infection obviously must play a rôle in regard to the percentage of animals in which the inoculation succeeds and a definite lesion results. In monkeys the percentage of successful inoculations is much higher than in rabbits, and the virus, while it sometimes becomes attenuated by successive passages through these animals, apparently usually does not become

[1] Mayer, Rocha-Lima, Werner: loc. cit.

[2] Cole: J. Cutan. Dis., N.Y., June, 1913, xxxi, 384.

[3] This experiment has since been published Trop. Dis. Bull., Lond., 1914, iv, 486.

FIG. 1. — DOG'S TESTIS. Showing experimental lesion produced by the inoculation of virus from a human being.

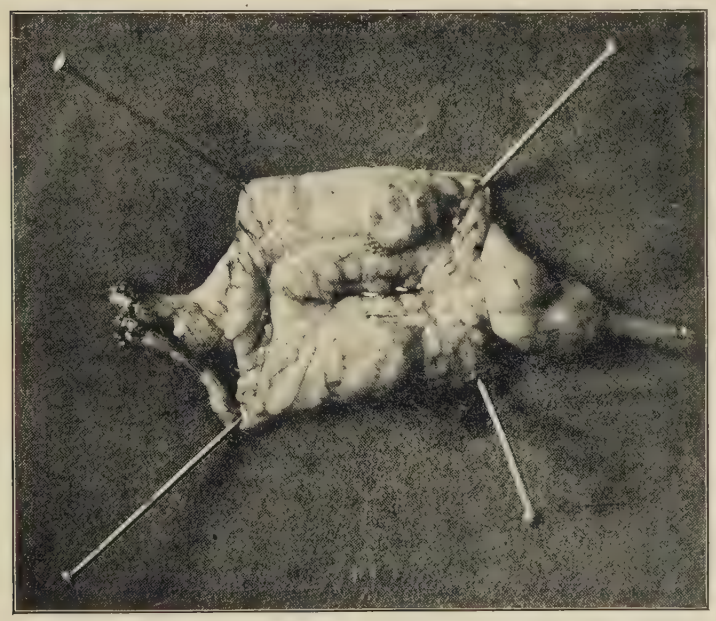


FIG. 2. — RABBIT'S TESTIS. Showing lesion produced by the inoculation of human virus.

PLATE XXVI. — VERRUGA PERUVIANA.

attenuated to the same degree as it does by successive passages through the rabbit's testis. When once the virus has been successfully transmitted from the human lesion to the monkey, it may be carried through many successive passages in these animals, as is shown in Table II.

Cultivation and Filterability of the Virus of Verruga

In many of the experiments already referred to in this section the attempts at cultivation of the virus of verruga from material obtained from the human lesions and from those produced in the rabbit's testis have been considered, and the results recorded. From these experiments it may be seen that even in those cultures, which suggested from their appearance the possibility of a growth, no definite microörganism could be demonstrated by microscopical examination or by subculture, and upon inoculation of some of these cultures into rabbits' testes no lesions were produced. While the results alone of the inoculation experiments into animals of this nature do not preclude a cultivation of the virus, since by this method of inoculation as we have seen from Table I only a small proportion of rabbits may be expected to develop lesions even when the fresh virus is employed, nevertheless, the results of these experiments do not give any support to the idea of a successful cultivation of the virus. Numerous attempts have also been made to cultivate the virus *in vitro* from the verruga lesions produced in monkeys by its inoculation. These experiments like those already considered in relation to the cultivation of the virus from the human and rabbit lesions have usually resulted in failure. Various modifications of the methods described by Noguchi and Murphy for cultivation have been employed. Great difficulty has been experienced in obtaining the material from monkeys free from bacteria. The monkeys almost invariably scratch and infect secondarily the lesions. It is not deemed advisable to consider in detail all of the experiments which have resulted in complete failure of cultivation of the virus from the lesions produced in these animals. However, the results will be

briefly referred to which were observed in a series of cultures made in ascitic fluid containing rabbit's testis to which had been added exudates produced by injections of aleuronat containing leukocytes, and small fragments of verruga nodules. In several instances such cultures showed certain differences from the control tubes, notably in a cloudiness of the media, and in the formation of a fine granular precipitate along the sides and bottom of the tube. This precipitate perhaps resulted from disintegration of the tissues in the culture by the growth of the virus. Apparently it did not constitute a true culture as examination revealed no bacteria or other visible microörganism, and subcultures on agar gave no apparent growth. In the case of one monkey, see Table II, inoculated with such a culture which had been incubated for sixteen days at 37° C., a small group of reddish papules appeared in a row on each brow after an incubation period of twenty-one days. These papules, however, never developed into as extensive lesions as almost invariably happens when the fresh virus is used, and they disappeared in a shorter time. Thus it appears that while the virus may have been alive in the culture media, it had evidently become more or less attenuated. Other attempts to infect monkeys with cultures have failed, as also may be seen from Table II. Experiments in cultivation were continued, but with no further success. The virus has become increasingly more and more attenuated in its successive passage through monkeys, and it has become increasingly difficult to obtain sufficient satisfactory material from which to prepare the cultures. The most satisfactory material for work in cultivation of the virus can only be secured directly from the human cases. The cultivation of the virus of verruga, then, seems to be today in a somewhat similar position to that of the virus of small-pox.

Filterable Qualities of the Virus

The experiments performed in relation to demonstrating the filterable qualities of the virus obtained from human lesions have been described previously. From this description, and

from Table I, it may also be seen that those rabbits inoculated with the diluted filtrates of suspensions made by grinding the human lesions in saline solution developed no lesions. These experiments are not conclusive, however, since as has been emphasized only a portion of the rabbits inoculated with the virus itself by the intratesticular method develop lesions. This fact is also shown in Table I, and the reason for this result has already been considered in this report. No monkeys were available for filtration experiments while human material was available. Although we had been informed that monkeys were plentiful in Peru, we had the greatest difficulty in obtaining them upon the western coast of South America, and it was necessary to import them via Panama. Subsequently, however, experiments were performed relating to the filterable qualities of the virus with material obtained from the lesions produced in monkeys by inoculation of the virus after numerous successive passages of it through these animals. In the experiments in which portions of the verruga nodules from monkeys were finely ground, suspended in water or saline solution, and the resulting mixture passed through Berkefeld filters (No. 2) the filtrates failed to produce lesions in monkeys, and monkeys inoculated with such filtrates were not rendered immune to subsequent infection with the unfiltered virus. These experiments are also summarized in Table II. Either the virus was present in too small amount in the filtrates, or was in too attenuated a condition to give rise to the production of lesions in an animal so relatively insusceptible to the infection as is the monkey, or else it had failed to pass through the pores of the filter. Nevertheless, we have been unable to detect, as has been shown in many of the experiments recorded, by microscopic examination either in numerous fresh preparations, often examined with the dark-field microscope, or in stained preparations made from the verruga lesions, or in cultures made from them, or in sections from the tissues, any definite visible microörganism. It seems not unlikely that the virus of verruga will ultimately be shown to be a filterable one.

As is well known, the success in filtering a virus or organism depends not only upon the form and dimension of it, and the texture and thickness of the filter used, but upon the pressure employed, the duration of the filtration, and the composition of the liquid in which the virus or organism is suspended. The results obtained in animals inoculated with the filtrates of some of the " filterable viruses " are frequently not constant, and before it can be definitely asserted that a virus is non-filterable, very extensive experimentation with a large amount of material is sometimes necessary.

For many years the virus of small-pox was regarded as non-filterable, and in many experiments made in connection with the filtration of this virus, the filtrates of it often fail to produce lesions in monkeys. Sims Woodhead [1] has recently again called attention to this fact and has mentioned that during an epidemic of small-pox he attempted to obtain vaccine by filtration but found that not a trace of active vaccine was present in the filtrate after the process was completed whether the dilution was high or low. Further experiments in relation to the question of the filterability of the virus of verruga should be performed with perfectly fresh material obtained from the human lesions, and the inoculation of the filtrates should be made into human beings or into monkeys to demonstrate whether the virus has passed through the pores of the filter in sufficient amount to produce lesions. Mayer, Rocha-Lima and Werner [2] have performed a single experiment regarding the filterability of the verruga virus. In this instance the filtrate from a Berkefeld candle of a suspension of four ground papules removed from their case was inoculated over the eyes of a monkey. The experiment resulted negatively, and no lesion appeared. We feel that the results of the experiments regarding the non-filterable qualities of the virus after its repeated passage through animals which have been performed upon monkeys should not be considered as conclusive. In connection with the consideration of the filter-

[1] Woodhead: Proc. xviith Internat. Cong. Med., Lond., 1913, Sect. 4, Bacteriology and Immunity, 122.

[2] Mayer, Rocha-Lima, Werner: loc. cit.

able qualities of the virus of verruga peruviana the important researches of Rous [1] relating to the nature of the filterable agent causing a sarcoma of the fowl are of great interest.

Similarity between the Virus of Verruga and of Small-pox

We have called attention to the fact that when monkeys are inoculated with the virus of verruga or that of small-pox, that only a modified form of the original disease results and in the case of verruga, only a localized lesion develops. Neither the virus of small-pox nor that of verruga, as we have shown, has been satisfactorily cultivated *in vitro* upon artificial media. In several other ways the virus of verruga shows some similarity to that of small-pox, but in other ways obviously the two behave very differently. So far no definite visible microorganism has been discovered in verruga peruviana, although Mayer, Rocha-Lima and Werner [2] in the study of a single case, reported the presence of bodies resembling chlamydozoa in the lesions. The cell inclusions observed in variola and vaccinia have also been referred by Prowazek [3] to the chlamydozoa. Schilling-Torgau [4] places both verruga peruviana and variola in the list of diseases caused by filterable viruses, but he gives no evidence to support the view that the virus of verruga is filterable. Apart from the evidence obtained from our own experiments and the single negative one performed by Mayer, Rocha-Lima, and Werner, already referred to on page 128, no experiments relating to this question apparently have been performed. The filterable properties of the virus of verruga have, however, already been discussed upon page 126. The virus of verruga is much less stable than that of small-pox and after the verruga virus from animals has been glycerinized even for a short time it is at least sometimes no longer inoculable for monkeys, as we have shown

[1] Rous: J. Am. M. Ass., Chicago, 1911, lvi, 198; 1912, lviii, 1938.

[2] Mayer, Rocha-Lima, Werner: München. med. Wchnschr., 1913, No. 14; Verhandl. d. deutsch. path. Gesellsch., März–April, 1913, 198.

[3] Prowazek: Handbuch der Pathogenen Protozoen, Leipz., 1912, Erster Band, 119.

[4] Schilling-Torgau: Mense, Handbuch der Tropenkrankheiten, Leipz., 1914, Zweite Auflage, Zweiter Band, 110.

(see Table II). The two viruses show similarity, however, in the fact that monkeys which have been successfully inoculated with either of them are subsequently usually immune to a second inoculation of the corresponding virus. The verruga virus, on the other hand produces no lesion when inoculated upon the rabbit's cornea, as does the variola one. The disease produced in man by the small-pox virus is of a more virulent type than that produced by the one of verruga.

The clinical appearance and structure of the lesions upon the skin are also entirely different and small-pox is very contagious. The inoculation of man with small-pox virus obtained directly from a human case of small-pox, however, is not usually a dangerous proceeding, and we have shown in one case that the inoculation of a man with verruga virus obtained directly from another human case was accomplished without serious result. After considering these facts it seems apparent that there is a slight analogy between the two viruses. However, the unique character of verruga peruviana perhaps impresses one as more remarkable than its similarity to any other disease. Reference has already been made to certain tumors of the fowl described by Rous as due to a filterable virus. (Page 129.)

Differential Diagnosis of Verruga Peruviana

Differentiation from Framboesia.— It has been suggested that verruga peruviana is but a form of framboesia. Thus Eder [1] inclines to the opinion that the two diseases, yaws and verruga, are nearly related, verruga being nothing more than a severe form of framboesia or yaws, modified partly by the high altitude of the regions where it occurs, and partly by being complicated with malaria. Biffi,[2] however, who has observed verruga peruviana in Peru is entirely opposed to this view. In our opinion, any one who has had a wide experience with either disease would not confuse, from a clinical standpoint, the one with the other. Only in the distribution of the lesions

[1] Eder: J. Trop. Med., Lond., 1906, ix, 213.
[2] Biffi: Arch. Schiffs- u. Tropenhyg., Leipz., 1908, xii, 1.

sometimes, and in the fact that in both small nodular tumors appear upon the skin, is there any resemblance in the two diseases. From the descriptions of the lesions already given on pages 65 to 81 this fact should appear evident. The most characteristic feature of the yaws lesion is that the primary one is a papule which after about a week becomes moist, developing a yellowish secretion which dries into a crust. The subsequent lesions are also commonly crusted. The verruga has an entirely different appearance. It is practically never crusted, as we have seen from the descriptions already given. Indeed, the most characteristic feature in its appearance is that the skin is smooth, tense, and translucent over it, and that the external surface of the lesion at the height of the disease resembles very much a cherry on account of its color and translucence.

Other points of differentiation between the two diseases which have been referred to are that framboesia is much more contagious than verruga. In framboesia there is generally a distinguishable point where the initial lesion developed. In verruga this is not so apparent. However, as we have shown elsewhere, there are other more decisive points of difference. Thus from an etiological and pathological standpoint the two are entirely distinct infections. In a very careful study of the lesions of verruga peruviana, as we have emphasized in the experiments discussed on pages 82–122, we have been unable to detect *Spirochaeta pertenuis* or any other visible microörganism. In the very numerous experiments in which fresh material from the human verruga lesions has been injected into the rabbit's testis, the lesions produced have not resembled those which result from the inoculation of material from the lesions of framboesia cases, and again no spirochaetae have been found in them. In the intratesticular lesion which results from the inoculation of framboesia material, as was first shown by Nichols,[1] spirochaetae are abundant. Finally, the disease verruga peruviana is not cured by injections of salvarsan, as was first shown by one of us (Strong)[2] in 1910, to be

[1] Nichols: J. Exper. Med., 1910, xii, 616.

[2] Strong: Philippine J. Science, Manila, 1910, v, 433.

invariably the case with framboesia. The lesions of the skin of verruga peruviana seem to be entirely uninfluenced by injections of this substance, while in framboesia they disappear very rapidly following its injection.

It is not deemed necessary here, to consider in detail the differentiation between the histological structure of the lesions of verruga and those of framboesia. In framboesia the lesions affect the epithelium rather than the cutis. The surface epithelium is greatly increased and the epithelial cells are degenerated and swollen. In verruga the most striking feature is the extensive endothelial proliferation occurring about newly formed blood-vessels, a condition which is not observed in framboesia. The histological appearances of the verruga lesions are thoroughly considered on page 145, and from this description it is evident that from a pathological standpoint, as well as from a clinical one, the two diseases are also distinguishable.

While discussing the differentiation of verruga peruviana from framboesia, it may be advisable briefly to refer to its differentiation from syphilis. From what has already been said regarding the absence of spirochaetae in not only the human lesions of verruga, but particularly in those produced in the rabbit's testis with verruga material, and from the complete resistance of the disease to treatment with salvarsan, it would appear evident that the disease is not a form of syphilis. The Wassermann reaction was negative with one exception in the cases of verruga which we studied. In performing these experiments cases of syphilis were used as positive controls, and normal cases for negative controls. In nine cases of verruga in which a well-marked eruption was present, the reaction of the serum was also investigated in relation to the fixation of complement with a specific antigen prepared from a verruga nodule. In none of these cases was a fixation of complement obtained. It is noteworthy in this connection that according to Castellani [1] and Bowman [2]

[1] Castellani: J. Hyg., Camb., 1907, vii, 566.

[2] Bowman: Philippine J. Science, Manila, 1910, v, 485.

fixation of complement occurs with the serum of yaws cases when yaws antigen is employed. Baermann [1] found that in yaws as in syphilis the Wassermann reaction is present.

In the study of the blood in verruga peruviana, it was found that an extract prepared from a cutaneous nodule from a case of verruga showed the presence of a hemolysin which was active in dilutions of 1 to 1,000.

Differentiation from Oroya Fever. — From the descriptions given in Sections III and IV, regarding Oroya fever and verruga peruviana, and from the experiments detailed in these sections, it would seem evident that Oroya fever and verruga peruviana are two distinct diseases. We believe that we have conclusively shown in these pages, first, that verruga peruviana is due to a virus which may be transmitted to several of the lower animals, particularly monkeys, in which animals characteristic lesions are developed; [2] second, that Oroya fever is due to a microörganism (*Bartonella bacilliformis*) which is a parasite of the red blood-corpuscles and of the endothelial cells. Up to the present time it has not been transmitted to animals. In man in severe infections this parasite gives rise to a rapid and intense anaemia which often results fatally. Verruga peruviana is very rarely a fatal disease, and there is no such marked anaemia accompanying it. Its most characteristic feature is the occurrence of the eruption upon the skin. When fresh material from the lesions of verruga is inoculated intravenously and cutaneously into monkeys, the parasite of Oroya fever does not appear in the blood of these animals. The pathology of the two diseases is also quite distinct, as is evident from the facts set forth regarding this question in Section III, page 42 and Section IV, page 137. *Bartonella bacilliformis* is not found in the blood of uncomplicated cases of verruga peruviana, but occasionally concomitant infections of Oroya fever and of verruga are encountered just as concomitant infections with verruga and malaria are frequently observed. This fact has been more fully discussed

[1] Baermann: München. med. Wchnschr., 1910, No. 41.

[2] The characteristics of this virus have been described on pages 122 to 130.

on page 64. In spite of the evidence which we had adduced of the specificity of the two diseases, many Peruvian physicians insisted upon their unity and claimed that in Carrion's experiment the inoculation was made with blood from a verruga nodule, and that he died of Oroya fever. On account of the insufficient data regarding this experiment, the condition present at the autopsy, and the examination of the blood, it is impossible to say today of what infection Carrion died. Certainly there is no definite proof that he died of Oroya fever. Nevertheless, it seemed advisable to repeat the experiment of Carrion. Having become convinced from clinical observation and experimental evidence that Oroya fever and verruga peruviana were two entirely distinct diseases, and having secured through the kind assistance of Dr. Matto, Professor of Bacteriology in the University of Lima, a volunteer (a Chilean), a direct inoculation was made from two cases of verruga peruviana. A portion of a skin lesion from each of the two patients with verruga was removed, and within twenty minutes of the time of this operation the skin of the normal person over the left shoulder was thoroughly scarified, and a portion of the nodules removed from the verruga patients thoroughly rubbed into the scratches. The vaccination scratches were healed entirely at the end of about ten days, and the skin appeared normal. On the sixteenth day two small groups of cherry-red papules at the end of some of the vaccination scratches appeared, and a few days later another small group became visible. At this time the note made regarding the experiment states:

"Today the papules measure from 1 to 2 mm. in diameter, they are situated in three clusters, at the ends of the vaccination scratches. The scratches have all healed and the skin appears otherwise normal. The papules are of a bright red color, raised above the surface of the skin, and sharply circumscribed. The skin covering them is tense and translucent."

These papules increased slowly in size until the thirty-fifth day, when two of the groups were removed. The blood examination of the vaccinated person never revealed the parasite observed in the blood of Oroya fever cases, and no appreciable anaemia developed. The red blood-corpuscles

numbered 4,300,000 per cubic mm. at the time of the inoculation, and at the time of the appearance of the eruption they were 4,500,000. On the day the papules were removed the red count was 4,216,000. The leukocytes numbered 10,000 on the day the papules were first observed. After a week the count showed 8,000. There was but a slight febrile reaction preceding the papular eruption and the symptoms preceding it were mild. The patient stated that he felt languid, and the facial expression gave the appearance of his being tired and depressed. No generalized eruption upon the skin occurred, and the individual was in good health at the time of our departure from Lima. A portion of the lesions removed from the case was placed in Zenker's solution for section, and another portion ground and suspended in saline solution, and inoculated into the testis of Rabbit No. 73. The sections of the lesions showed distinct papular formation and a similar histological structure to that observed in other early human lesions of the disease. The rabbit developed a typical verruga lesion in the testis after twenty-five days. From this experiment it appears evident that the direct inoculation of the verruga virus from man to man does not produce Oroya fever, but a modified form of verruga eruption, just as the inoculation of small-pox virus from man to man produces a modified form of small-pox eruption. From this experiment it would also appear that the last objection to the idea that verruga peruviana and Oroya fever are two distinct diseases as based upon Carrion's experiment has been removed.

Relationship to Paratyphoid Fever.— It has been suggested that Oroya fever is paratyphoid to which yaws is superadded. This statement, it appears to us, requires no further discussion here. We have already referred to the fact on page 39 that paratyphoid fever has often been diagnosed in Peru under the name of Carrion's fever, and sometimes as Oroya fever. Of the twenty-four cases of verruga peruviana which we studied, none of them were complicated with paratyphoid infection. Of course there is always a possibility that a concomitant infection with these two diseases might occur. During the

time our investigations in Lima were being made, we observed a case of fever without any eruption which was shown to us as Oroya fever. We found no microörganisms in the red blood-corpuscles of this patient. Subsequently we were able to show by bacteriological examination that the case was one of paratyphoid infection, and not one of Oroya fever.

From the discussion given in this section of the report it seems evident that verruga peruviana is a distinct disease *sui generis*.

Relationship to Angiofibroma Cutis Contagiosum. — Bassewitz [1] has described in Brazil under the name of " angiofibroma cutis circumscriptum contagiosum " a disease which from the description given evidently bears considerable resemblance to verruga peruviana. Bassewitz states that from the appearance of the lesions upon the skin alone a differential diagnosis of the two is difficult. No spirochaetae were found in the lesions. He believes, however, that this angiofibroma is a different disease from verruga, basing his views upon the descriptions given in the literature regarding the symptoms of verruga peruviana. He calls attention to the fact that there is not the same high mortality in angiofibroma contagiosum, that the eruption is not preceded by high fever, anaemia, and prostration, and that symptoms due to the development of the lesions internally also do not occur. Rocha-Lima [2] in a more recent publication gives these same views as his own although he has never apparently had an opportunity of examining the fresh blood in a case of Oroya fever and has studied but one case of verruga peruviana. We have pointed out above that these symptoms do not occur in uncomplicated verruga peruviana, and the mortality of the disease is not high. From a histological as well as a clinical standpoint it appears that the two conditions if not identical are probably very closely related. The histology of both is considered on page 151. Further information, however, is desirable before verruga peruviana and angiofibroma circumscriptum contagiosum can definitely be regarded as one and the same disease.

[1] Bassewitz: Arch. f. Schiffs- u. Tropenhyg., Leipz., 1906, x, 201.

[2] Rocha-Lima: loc. cit.

Pathology

The distribution and description of the lesions as they occur upon the skin and visible mucous membranes have already been referred to at some length on pages 65 to 81. When the smaller papular lesions are removed, the tumors are seen to be rounded or oval. The cut surface is very moist, and serum and a variable amount of blood escape as the section is being made. In the more acute lesions the cut surface of the superior portion of the papule usually is of a bright cherry-red color, while at the base the tissue has a grayer appearance. Under a hand lens the cut surface of the papule appears moist, smooth, homogeneous, and very translucent. No blood-vessels of sufficient size to be recognized with a hand lens are present, and no lobular appearance is observable.

Some of the larger lesions measuring from 1.5 to 2 cm. in diameter, after removal present a different appearance. These may be rounded or dome-shaped. In the so-called mulaire lesions the skin covering the nodule sometimes resembles somewhat an onion skin. This appearance is given by the fact that the superficial layers of the skin have become slightly dry and the surface has become traversed by multiple longitudinal lines. These lesions after removal usually present a variegated appearance, being bluish, grayish, or purplish in color. On cut section through the middle of the larger tumors the surface is also moist, and serum and blood exudes. The cut surface in general is dark cherry-red, traversed by longitudinal gray lines. Sometimes yellowish-gray areas are seen in the substance with gray lines radiating about them. Under the microscope newly formed connective tissue is seen to occupy the areas in which the gray lines are present, and evidently gives rise to the appearance described. The yellowish-gray areas are seen microscopically to comprise the more compact cellular portions of the nodule. A few very small blood-vessels can be distinguished with the naked eye in the larger lesions. Sometimes the skin has become quite adherent over the tumor, and in the cut section in these instances some

of the gray lines radiating from the surface evidently represent an invasion of epithelium into the depth of the tumor for a distance of several millimeters. The larger mulaire lesions sometimes show superficial ulceration and present then a reddened, moist surface. The appearance of these lesions is obviously somewhat modified by secondary infections with bacteria. Deep ulceration practically never occurs.

The distribution of the lesions upon the skin and visible mucous membranes, the lips, gums, conjunctivae and glans penis has already been described.

In the description of the disease given in the textbooks, one usually finds the statement that the eruption occurs in the internal organs. Thus Scheube [1] writes,

> "There exists no mucous membrane or organ in which they may not appear, and the symptoms they can originate are remarkably manifold. Thus excrescences in the nose may cause epistaxis; in the throat and oesophagus, dysphagia; if situated in the larynx, cough, hoarseness, haemoptysis, and even suffocation may result; if they have their seat in the lungs haemoptysis and infiltration may set in, which may be mistaken for tuberculosis; when situated in the stomach they cause haematemesis; in the intestine, diarrhoea and intestinal haemorrhage; in the kidneys or bladder, haematuria; and in the uterus, metrorrhagia. In rare cases the brain and spinal column participate in the disease, inducing epileptiform convulsions and symptoms of meningitis."

The idea of the occurrence of the eruption in the internal organs appears to have been passed on in the literature from author to author since 1845, when Tschudi [2] in addition reported the occurrence of the lesions in the bones. In our opinion the evidence given regarding the occurrence of the lesions of verruga peruviana in the internal organs is not conclusive. The anatomical changes that have been found in the solid viscera in this disease appear frequently, at least, to have resulted from complication with other diseases. Thus it is evident that the internal lesions of tuberculosis have sometimes been mistaken for those of verruga peruviana. The tissues of the organs from Cases B and X, reported upon on page 54, in which the diagnosis of Oroya fever and verruga peruviana had been made during life by the physicians who observed them, evidently came from individuals suffering

[1] Scheube: loc. cit., 302.

[2] Tschudi: loc. cit.

with tuberculosis. This fact we were able subsequently to show from a microscopical study of the sections of these organs. We were also able to demonstrate acid-fast bacilli in these sections. During our stay in Peru in several instances, miliary tubercles in the spleen and liver, due to tuberculosis, were shown us by physicians as examples of the internal lesions of verruga peruviana. Attention already has been called to the fact that Nicolle, Letulle, and Galli Valerio found acid-fast bacilli in the lesions of verruga, and that Darling observed at autopsy a case of verruga with generalized tuberculosis with lesions in the lungs, pleurae, lymph-nodes, spleen, kidneys, and liver. Nicolle [1] in a case diagnosed as *maladie de Carrion* with internal verruga lesions found acid-fast bacilli resembling the tubercle bacillus in lesions in the liver, lungs, spleen, and lymphatics. Giant-cells were observed in many of the tubercles. Bindo De Vecchi [2] studied microscopically the tissues from two cases of verruga peruviana said to have shown at autopsy internal lesions. The tissues were sent to him from Peru. In the first case which had high fever during life a lymphatic abscess was said to have been present from which a paracolon bacillus was isolated. Regarding the second case no definite clinical information was furnished. In both of these cases in addition to the skin lesions De Vecchi found lesions in the liver, spleen, and lungs. These internal lesions consisted of nodules showing necrosis with formation of fibroblasts and giant-cells. The pictures of the internal lesions in the liver and spleen which De Vecchi publishes are very different from the verruga lesions as we have observed them. He also observed bacilli present in many of these lesions. During our stay in Lima, through the kindness of several Peruvian physicians, and particularly through that of Dr. Hercelles, we had opportunity of examining all of the pathological material present in the museum of the Dos de Mayo Hospital relating to verruga peruviana and Oroya fever. None of the lesions we observed in the internal organs

[1] Nicolle: loc. cit., 593.

[2] De Vecchi: Beiheft 4, Arch. f. Schiffs- u. Tropenhyg., Leipz., 1909, xiii, 143. Virchow's Arch. f. path. Anat., Berl., 1908, cxciv, Beiheft, 1.

were convincing that they were of a verruga nature.[1] Only in two of the cases did it seem that there was any question as to whether the lesions were those of verruga peruviana. In one of these a number of small tumors, measuring about 4 mm. in diameter, were situated in the heart beneath the pericardium, and in another specimen from another case there were small miliary nodules in the pharynx and larynx. A description of the histological examination of the sections from these lesions has been given on page 53. Microscopical examination of the one from the heart showed multiple cysts caused apparently by a cestodal infection. In sections of the other specimen from the pharynx numerous rounded masses of vascular tissue were present. There apparently was no tendency to caseation and no giant-cells were discovered. No acid-fast bacilli were found in the lesions. It seems probable that these lesions in the pharynx and larynx represent those of verruga peruviana, and that the eruption of the disease occurred not only in the mouth, but also in the pharynx and upper part of the larynx. No lesions were found in sections of the spleen from this case.

Histology

In examining the literature we find that the histology of the verruga nodules of the skin has been studied by the following investigators.

Previous Investigations. — Velez[2] expressed the opinion that the cutaneous verruga nodules originated in the papillary layer of the skin and mucous membranes. Dounon, Cornil, and Renaut[3] from the study of sections of the nodules concluded that they were of a fibrosarcomatous structure, being composed largely of embryonic connective tissue.

Izquierdo[4] described the connective tissue structure of some of the tumors, and noted their resemblance to sarcoma.

[1] In connection with this statement reference may be made to the review of Hercelles' article (Trop. Dis. Bull., Lond., 1914, Dec. 15, iv, 486) which has appeared while this report was in press.

[2] Velez: Gac. méd. de Lima, 1861, v, 198.

[3] Dounon, Cornil, Renaut: Arch. de méd. nav., Par., 1871, 255.

[4] Izquierdo: Virchow's Arch. f. path. Anat., Berl., 1885, xcix, 411.

He emphasized the vascularity of some lesions and mentions a true cavernous structure in some portions of others. He points to the great resemblance of these to the true cavernous angioma. He also noted the presence of non acid-fast bacilli in the sections.

Letulle [1] observed subacute proliferation of the dermic and subcutaneous tissues, together with hyperplasia of the endothelial cells. In places the specific elements of the skin had disappeared entirely. The arteries and nerves remained intact. Other areas composed of lymphoid-cells surrounded by a thin endothelial wall were also noted. Acid-fast bacilli were found in all of the lesions.

Nicolle [2] examined histologically fragments of liver, kidney, lungs, spleen, and lymphatics, said to have come from a case of the Oroya fever type in which there were only internal verruga lesions. In the lungs epithelioid nodules surrounded by leukocytes were present. There was no caseation and no giant-cells were observed. In the liver, however, giant-cells were found, but no nodules and no necrosis. The lymphatic glands showed caseation, and similar lesions were found in the spleen. Acid-fast organisms resembling the tubercle bacillus, from a morphological standpoint, were found in these lesions.

Hercelles [3] attributed a vascular origin to the nodes, believing that they resulted from a periarteritic proliferation. The regression of the lesion was thought to be due to an endoarteritic process.

Sometimes giant-cells were observed in the sections. He believed the substance of the verruga nodules to be formed from a reaction which resulted in the massing together of the tissue cells.

Escomel [4] believes that the organism of verruga is carried in the blood where it secretes its complex toxin. It is inter-

[1] Letulle: Mém. Soc. de biol., Par., 1898, 764.
Odriozola's Monograph, 201.
[2] Nicolle: Ann. de l'Inst. Pasteur, Par., 1898, xii, 590.
[3] Hercelles: Thèse de Lima, 1900.
[4] Escomel: Ann. de dermat. et syph., Par., 1902, iii, 961.

rupted at points where the circulation is slow and where there is a favorable medium such as the skin for its development. Leukocytes accumulate at this point, and a proliferation of the connective-tissue cells occurs. Later an increased number of newly formed blood-vessels appears. The typical verruga nodule is said to consist of verruga cells, "cellules verruqueuses" (formed from the connective-tissue cells), few leukocytes, and a connective tissue framework. In some lesions many red blood-corpuscles, leukocytes, and blood pigment were present. In the regressive ones the leukocytes were not abundant. He believes the verruga nodule belongs in the inflammatory group of lesions rather than among the true tumors. Escomel also found bacteria present in many of the lesions which he studied.

De Vecchi,[1] as has been mentioned on page 139, studied tissues sent to him from Peru which had been removed from three cases. The third of these was apparently a case of Oroya fever, and has been considered on page 52 of this report. No verruga nodules were found in the internal organs. The first case was complicated with pyogenic infection. There was high fever during life and swelling of the lymphatic glands. At autopsy the spleen was greatly enlarged, and in the submaxillary glands an abscess containing pus was present. There were also nodules in the skin and muscles, and tubercles in the lungs, spleen and liver. A paracolon bacillus was said to have been isolated from the pus of the abscess. Bacteria were found in the sections of the internal lesions by De Vecchi. These he refers to as bacilli of the typhoid-colon group. In one case De Vecchi describes the lesions in the spleen as multiple necroses. Multinuclear giant-cells were present which he states resemble the giant-cells seen in tuberculosis. Some fibroblasts were present. De Vecchi does not believe, however, that the lesions were produced by the tubercle bacillus. Similar changes, but not so advanced, were found in the liver. In the absence of animal inoculations the non-

[1] De Vecchi: Beiheft 4, Arch. f. Schiffs- u. Tropenhyg., Leipz., 1909, xiii, 143. Virchow's Arch. f. path. Anat., Berl., 1908, cxciv, Beiheft, 1.

tubercular character of these lesions is to be questioned. In the lungs neither degenerative lesions nor giant-cells were observed, but areas were present in the center of which the nuclei stained poorly. The reader should consult De Vecchi's article for the full description of these changes. Tubercle bacilli were not found in the lesions, but other bacilli single and in masses were present. In the second case it is stated that necrotic changes were present in the spleen, though of slight degree. The nodules in the liver De Vecchi characterized as typical of verruga lesions. Lesions were also found in the lungs which were partly necrotic, and a bronchial pneumonia was present. In the liver and spleen, bacilli were found, and in the lesions of the lungs, cocci were in addition present. Acid-fast bacilli were not found. In the lesions of the skin and muscles the degenerative changes were not recognized, and giant-cells were not observed. De Vecchi believes that the lesions in the beginning are simply of a hemorrhagic nature, and that later in such areas there is a proliferation of the connective tissue elements. He also believes that the necroses in the liver and spleen result from other hemorrhages from the newly formed blood-vessels. The most striking characteristic to him, regarding the verruga lesions, was the presence of "globulifere" cells,— cells formed by the leukocytes having engulfed red corpuscles. He also refers to the occurrence of "acidophile" bodies, measuring from 1 to 2 μ in diameter which he believes probably originate from hemoglobin. Blood-corpuscles which often stained poorly were observed near these areas. The verruga nodule he regards as of fibroblastic structure, a product of preëxisting elements in the tissue and the expression of the reaction of the tissue to an abnormal stimulus. A small number of mono- and polymorphonuclear leukocytes was observed in the lesions. Newly formed blood-vessels were almost invariably present in the smaller papules.

More recently Rocha-Lima[1] had the opportunity of studying histologically lesions from the skin from a case of verruga peruviana observed in Hamburg with Mayer and Werner.

[1] Rocha-Lima: Verhandl. d. deutsch. path. Gesellsch., Jena, 1913, 198, 409.

He employed modern methods of technique, and his description of the histology of the disease in our opinion is the most accurate which has yet been given. He points out that the compact cell masses observed in the lesions are composed of the proliferated endothelial cells from the newly formed blood-vessels, and that these cells should be designated as angioblasts. No giant-cells were found. In spite of the superior technique, and of employing various staining methods, which included Romanowsky-Giemsa, Gram, and Levaditi methods, as well as tubercle bacillus staining, he was unable to observe either bacteria or protozoa in the lesions. On the other hand, within many endothelial cells as well as in the proliferated angioblasts from the blood-vessels, he found conglomerations of fine granules which were not sharply bounded, and that were clearly visible only in the sections stained by Giemsa's and Levaditi's methods. The position, size, form, and general appearance of these structures reminded the author greatly of the bodies observed in trachoma, molluscum contagiosum, birdpox, and other diseases in which "chlamydozoa" have been described. No proof whatever that the granules Rocha-Lima observed in the degenerating cells were parasites is given. It seems remarkable that he should draw such sweeping conclusions regarding the etiology of this disease from the study of one case observed late in the course of the disease.

Finally Cole [1] studied histologically the lesions of a case of verruga observed in Jadassohn's clinic in Berne, and also those produced in monkeys by inoculation with verruga material. He concludes that "as the other granulomata — tuberculosis, syphilis, sporotrichosis, actinomycosis, etc., have their own significant histological changes, so also does verruga peruviana, belonging to the same class, have its own characteristic microscopical picture. It is characterized by a dilatation of the lymph vessels and a choking of their lumina with mono- and polymorphonuclear leucocytes; also by an infiltration around these vessels of plasma cells, fibroblasts, mononuclear

[1] Cole: Arch. internal med., 1912, x, 668.
J. Cutan. Dis., N. Y., 1913, xxxi, 384.

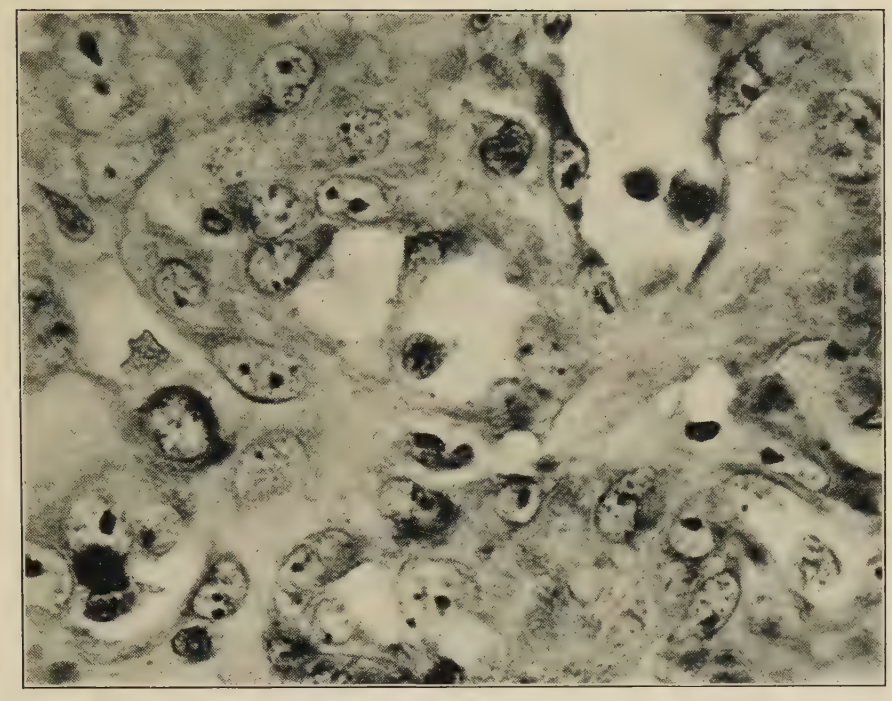


FIG. 1. — SECTION OF SKIN LESION.
Showing proliferation of angioblasts about vessels.

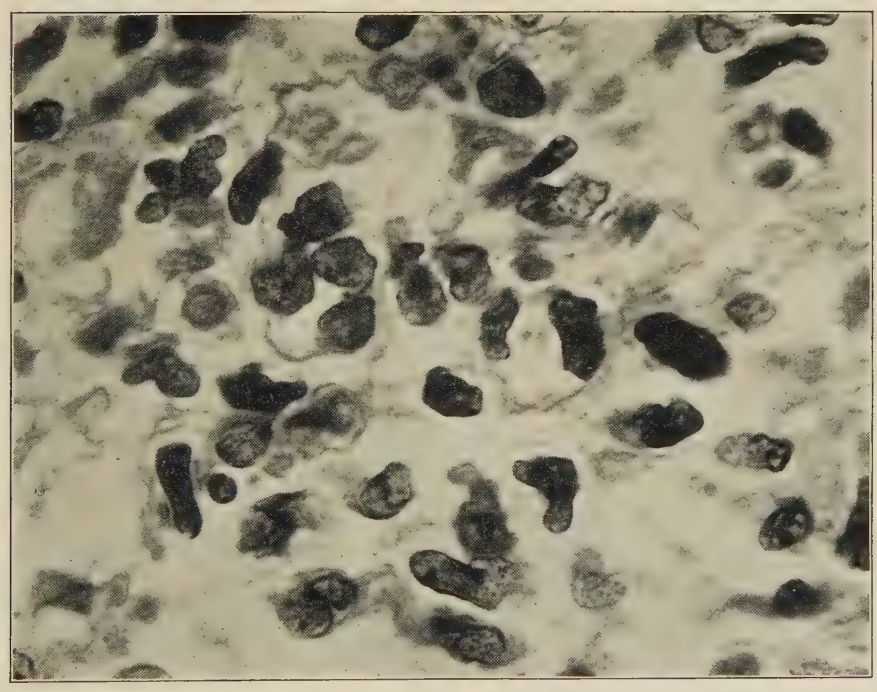


FIG. 2. — VERY EARLY SKIN LESION. Showing endothelial proliferation about vessels.

PLATE XXVII. — VERRUGA PERUVIANA.

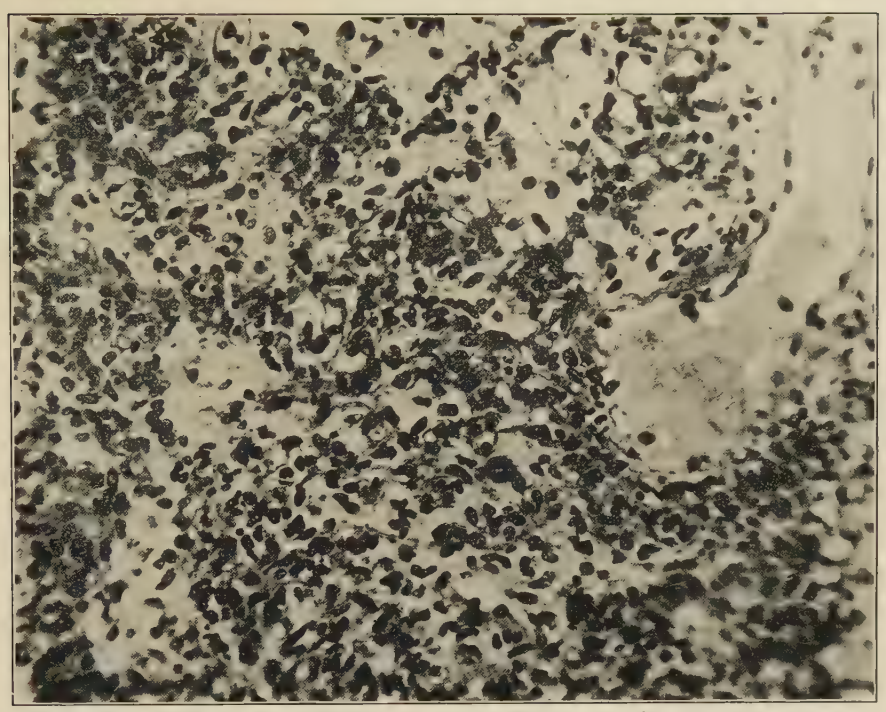


FIG. 1. — EARLY SKIN LESION. Showing very vascular structure.

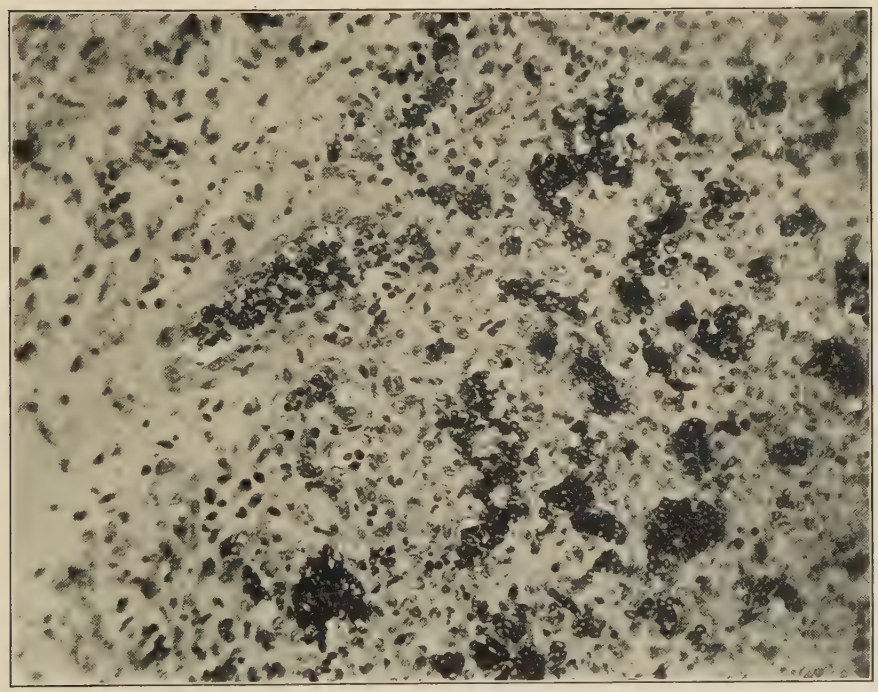


FIG. 2. — SKIN LESION. Section stained with phosphotungstic acid stain to show vascularity.

PLATE XXVIII. — VERRUGA PERUVIANA.

leucocytes and relatively small numbers of polymorphonuclear leucocytes. It is further characterized by the formation and dilatation of a great number of blood capillaries and by an extravasation of much serum and many red blood cells into the tissues. The lymph vessels either rupture at an early stage or dilate to large dimensions when their cellular contents undergo a pycnotic degeneration and hyaline change, with destruction of the vessel and invasion of the mass by plasma cells and fibroblasts." The tumors examined from both the patient and the monkeys resembled each other so closely in their histological structure and mode of formation, that Cole did not describe them separately. No giant-cells were found in the preparations. Neither in smear preparations from the tissues nor in stained preparations of them was any parasite or organism found except secondary invading cocci and bacilli. Gram's, Gram-Weigert's, polychrome methylene blue, tubercle bacillus stains, Mallory's eosin, methylene blue, Mann's, von Krogh's method with polychrome methylene blue, and Giemsa's stain were used. Cole does not refer to the presence of " chlamydozoa," but he calls particular attention to large cells lying in the center of the surviving lymph vessels which show signs of degeneration. In these the chromatin granules are first massed together in the nucleus, then deeply staining masses are seen in the protoplasm, while the latter begins to show signs of vacuolization and hyaline change, and the nucleus disappears. Finally the cell outlines themselves disappear and only the masses of degeneration products and hyaline are left. The endothelial wall about these large, degenerating, lymph areas remains intact at first, but later disappears when the surrounding fibroblasts begin to penetrate the mass.

Present Investigations. — During the time our studies in Peru were being carried on we collected a large amount of perfectly fresh verruga lesions of the skin for histological study. Lesions in the different stages of the disease from the very early to the very advanced ones were obtained. In this way we were able carefully to study the manner in which the lesions

develop. The tissues were hardened in Zenker's solution, in corrosive sublimate and alcohol, and in Fleming's solution, or in formalin. Various stains were employed including Wright's, Romanowsky-Giemsa's, Gram's, Levaditi's, haematoxylin, eosin, methylene blue and eosin, Mallory's connective tissue and phosphotungstic haematoxylin stains, *scharlach Roth*, acid fuchsin, and the tubercle bacillus stain.

We shall now attempt to give a general account, based upon the study of the tumors from the different cases, of the histological appearances of the lesions, showing the changes which they undergo as they progress.

The early lesions consist of newly formed blood-vessels lying in connective tissue which as the lesion progresses in size becomes oedematous. The oedematous areas, when developed, are often very poor in cells, though at different stages of the lesion a variable number of lymphocytes, larger plasma-cells, and polymorphonuclear leukocytes are present. The early papule is rich in blood and free blood-cells are usually present. The newly formed blood-vessels are often very numerous, and a striking feature in regard to many of them is the small calibre of the vessel in comparison to the number and large amount of protoplasm present in their endothelial cells. (Plate XXVII.) In some instances the endothelial cells of the vessels form more than one layer, those on the outside continuing to proliferate. Sometimes the appearance suggests that a capillary vessel has become occluded, and swelling and proliferation of the endothelial cells has resulted. The nuclei of these cells sometimes show mitosis. As the lesion progresses there is a very extensive proliferation of these angioblasts which give rise to large islands of closely placed cells in which the lumina of the small blood-vessels become compressed and no longer visible. Only a few fibrils can be detected sometimes between the cells. In these areas and about their periphery small numbers of true fibroblasts may be made out. The prevailing type of cell in the early verruga nodule is the angioblast. Other writers with the exception of Rocha-Lima and Cole, as we have intimated, refer to the prevailing type of cell

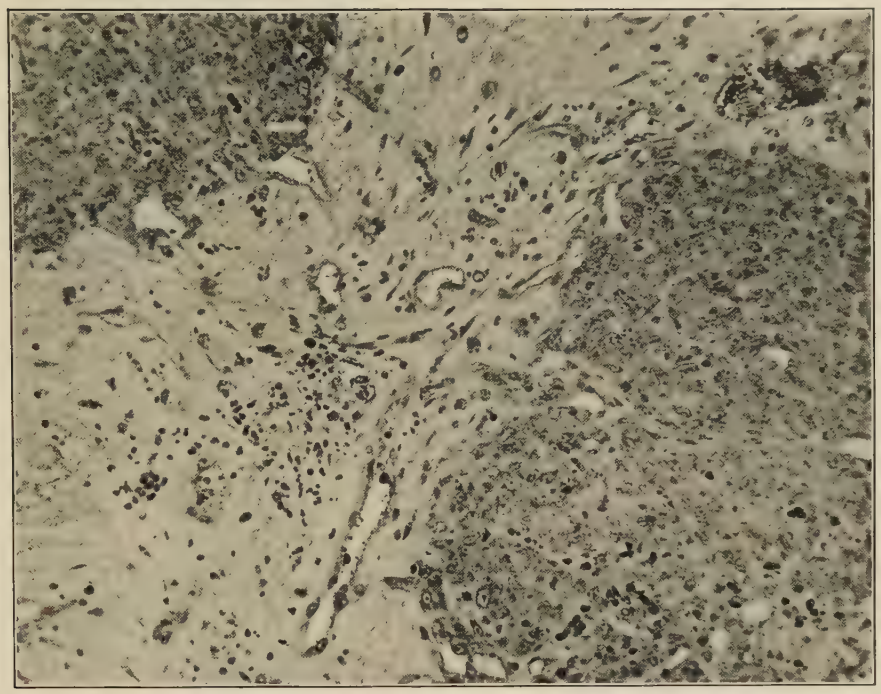


Fig. 1. — Nodule of Skin. Showing islands of closely placed angioblasts, oedematous connective tissue, and numerous vessels.

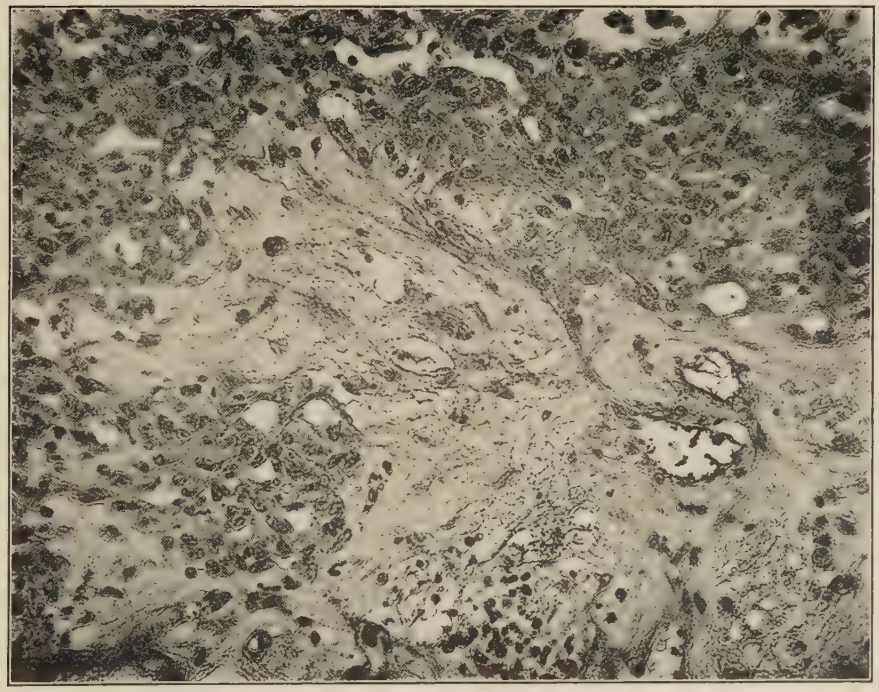


Fig. 2. — Nodule of Skin. Showing islands of angioblasts, oedematous connective tissue, and numerous vessels.

Plate XXIX. — Verruga Peruviana.

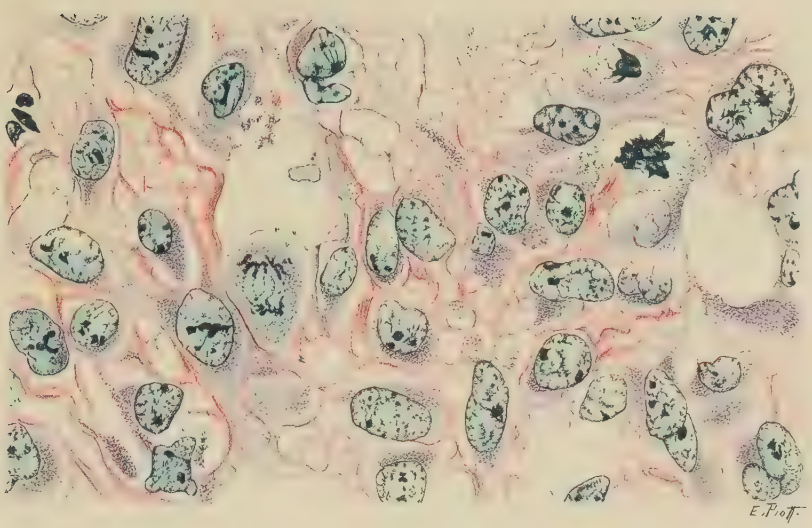


Fig. 1

Section of nodule showing particularly mitosis.

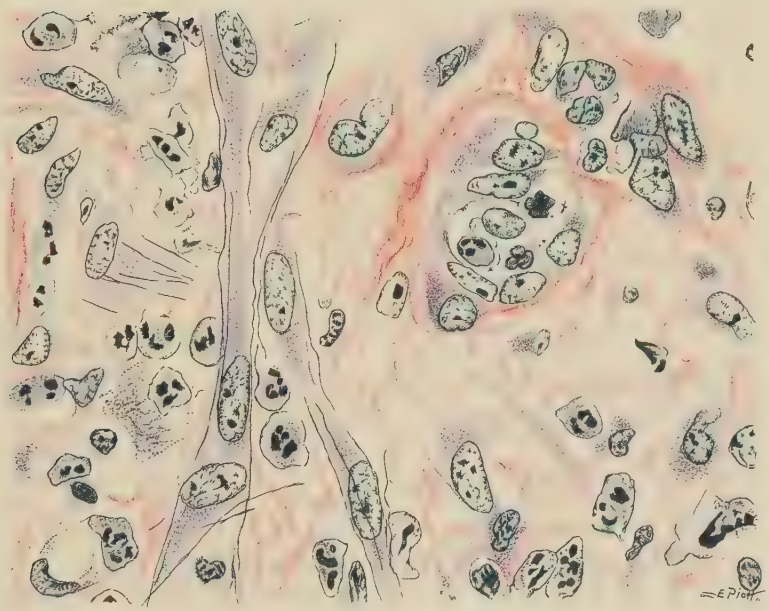


Fig. 2

Section showing proliferation of angioblasts,
and appearance of fibroblasts.

PLATE XXX — VERRUGA PERUVIANA

in the verruga nodule as the fibroblast. Dounon and Izquierdo emphasize the fact that the nodules consist of a structure resembling sarcomatous tissue. Escomel assigns a specific character to these cells and designates them as verruga cells. Bindo De Vecchi insists that they are merely fibroblasts, while Cole describes the prevailing type as the plasma-cell. A careful study of the very early lesions, however, seems to convince one that these cells are true angioblasts, a view also held by Rocha-Lima. This seems even more clear when one studies the staining reactions of these cells and compares these reactions with those of the endothelial cells of the small blood-vessels, and also studies the progress of development of the lesions. In no other condition does one find such a striking and so characteristic a proliferation of the endothelial cells lining the blood-vessels, as is encountered in the early verruga nodule, and it is this feature which particularly distinguishes the lesion from other pathological processes. Mitotic figures are numerous among these angioblasts. (Plate XXX.) In the older lesions the fibroblasts have gradually invaded the islands of angioblasts and deposited collagen fibres between them. (Plate XXX.) In this way the nodules come more closely to resemble a fibrosarcomatous structure. (Plate XXXI.) The verruga nodule, therefore, constitutes a special form of granuloma characterized in the early stages by the formation of new blood-vessels in oedematous connective tissue, and by marked proliferation of the angioblastic cells forming masses or islands of closely placed cells, by the invasion of the connective tissue by lymphocytes, plasma-cells, and leukocytes, and as the lesion progresses, by the formation of fibroblasts and the deposit of collagen fibrils. (Plates XXVII to XXXII.)

The amount of connective tissue varies greatly in the different lesions, and also its characteristics. Sometimes elastic fibers may be demonstrated by appropriate staining methods. The number of long spindle cells is greater in the older nodules, giving them more a fibrosarcoma-like appearance. The number and size of the blood-vessels, and also the number of free blood-cells varies in the smaller and larger lesions. The

amount of oedema is usually greater in the larger lesions. In the subcutaneous nodules, however, the lymphocytic infiltration is more marked. In the so-called mulaire lesions the epithelium has often disappeared and the surface is covered with a more or less organized blood-clot in which a variable amount of fibrin, polymorphonuclear leukocytes, and round cells are present. Bacteria are also often present. Such lesions beneath the extravasation of blood often show a cavernous structure. In these areas the oedematous connective tissue is penetrated by thin-walled blood-vessels of variable size. Often small extravasations of blood have occurred in these areas.

Thus, in the different lesions sometimes the condition found resembles somewhat a fibrosarcoma, sometimes a myxosarcoma, and sometimes an angioma. This seems to explain in part the differences in the descriptions of the lesions which have been given by other investigators. Apparently none of the previous histological studies have been based upon a very large amount of material. Usually only a small amount of material from single cases has been studied, or the report has been made from the study of a few pieces of tissue removed from a case which has occurred at a distance.

Giant-cells were observed in only one of the lesions which we have studied. This was evidently a very old lesion in which strands and islands of cornified epidermis were intermingled with tissue constituting the nodule. There were in this instance foreign body giant-cells grouped around islands of disintegrating epithelium. This may explain other observations relative to the presence of giant-cells in verruga lesions, although the frequency with which tuberculosis has been mistaken for verruga has already been shown. Plasma-cells were not found in the granulomatous areas, but when present were seen more particularly at the periphery of the lesions in some instances lying in the oedematous connective tissue. Mast-cells were occasionally observed in the sections. The prevailing cell in the cell nests described above as constituting the compact portions of the nodules, in the active phase, as we

FIG. 1. — SECTION STAINED WITH EOSIN AND METHYLENE BLUE. × 10.

FIG. 2. — SECTION STAINED WITH MALLORY'S CONNECTIVE TISSUE STAIN. × 10.

PLATE XXXI. — VERRUGA PERUVIANA. Lesions of the skin.

Fig. 1. — Section stained to show connective tissue.

Fig. 2. — Section showing cellular structure.

Plate XXXII. — Verruga Peruviana. Lesions of the skin.

have emphasized, is the angioblast. These cells are more or less flattened; the nucleus is large, vesicular, oval in shape, and does not stain deeply, being only moderately rich in chromatin; it often shows mitosis. These cells are sometimes found in various stages of degeneration, and are often vacuolated. They are the elements which Escomel refers to as "cellules verruqueuses," and which Cole refers to as cells of the lymph capillaries or plasma-cells. Numerous phagocytic endothelial cells sometimes containing red blood-corpuscles and cellular débris are often frequently observed in the tissues. These were described by De Vecchi as the "globulifere" cells.

As we have called attention to in other portions of this report, no visible microörganism has been detected in preparations made from the unbroken nodules of the skin and in sections of the tissues. In those lesions in which the skin had become abraded bacteria and a few blastomyces have been found in the superficial portions of the lesion. Many degenerating endothelial cells have been encountered in the sections, the phagocytic cells showing at times extensive granulation, and with nuclei in various stages of degeneration. Mitotic figures, as emphasized, are also frequent in other cells. No bodies which definitely could be identified as protozoa or other microörganisms have been detected in the cells. The granular endothelial cells were much more commonly encountered in the lesions from monkeys than in those from human cases.

Lesions in Animals. — The verruga lesion which develops as the result of the inoculation by scarification of the eyebrows of the monkey resembles closely that of the human being. The earlier lesions removed about six days after their appearance consist of a mass of vascular tissue showing a high degree of endothelial proliferation. The blood-vessels are irregular and tortuous and are for the most part considerably larger than the normal capillaries. The endothelial surface of these vessels is somewhat irregular from projecting cells. These cells, however, are not wholly confined to the lining of the blood-vessels, but are more or less distributed through the

tissue. Mitotic figures are numerous among them. They frequently show in the cytoplasm an area staining with eosin and in some instances this is large and prominent. To us, this appearance seems to represent an abnormality in the region of the centrosome. Whether it is the structure regarded by Meyer as a chlamydozoön, is not known. There is a small amount of extravasated blood in the tissue between the vessels, and a greater degree of infiltration with polymorphonuclear leukocytes than is usually seen in the human lesions. The skin overlying this mass of tissue appears as though somewhat stretched in the earlier stage of growth but otherwise shows nothing abnormal.

Lesions removed about two weeks after their first appearance show a considerable number of collagen fibrils intermingled with the vascular tissue. The leukocytic infiltration is now much more pronounced, but the blood-vessels showing a thickened and irregular endothelium still persist. The overlying epidermis shows ulceration and sometimes marked changes probably of a secondary origin and nature. There is marked down-growth of the epithelium in places and considerable hydropic degeneration with the production of microscopic vesicles. Phagocytosis at this stage of the lesion is a prominent feature, and cells are observed distended with rounded bodies probably of the nature of cellular débris. A considerable proportion of the endothelial cells show rounded hyaline eosin stained inclusions.

The verruga lesion of the rabbit's testis produced by the inoculation of verruga virus shows certain points in common with the human lesion, but in the stages in which it has been observed it differs more from this than does the lesion of the monkey. The lesions are multicentric and this is evidently due to the manner of the distribution of the virus by being inoculated in suspension. Proliferation of the blood-vessels is present, although it is a much less prominent feature than is observed either in the lesions of the human being or of the monkey, being overshadowed by the exudative process which is the most prominent feature. The central portion of the

lesion consists of necrotic tissue infiltrated throughout with leukocytes, a large proportion of which are disintegrated. Around these is a zone of vascular tissue through which the leukocytes are migrating. At the periphery of the lesion areas are observed infiltrated chiefly with lymphoid and plasma-cells and with smaller numbers of polymorphonuclear leukocytes. However, the greater part of the tissue is not composed of proliferating blood-vessels as in the lesions of the monkey. A few giant-cells are seen but these are chiefly around masses of pigment which may have been introduced with the inoculated material. An old lesion removed forty-seven days after inoculation and somewhat over five weeks after it first became palpable shows chiefly foci of lymphoid cells in a mass of fibrous tissue. All evidence of vascular proliferation has at this time disappeared.

Hence lesions of both the monkey and the rabbit show a proliferation of the blood-vessels, but the lesion of the monkey resembles much more closely the human one. In the monkey there is considerable infiltration with leukocytes and this process increases as the skin becomes abraded through the rubbing of the lesions. In the rabbit, on the other hand, the cellular infiltration and foci of necrosis constitute a more prominent feature than the vascular proliferation.

Angiofibroma Circumscriptum Contagiosum.— Reference has been made to the disease described in Brazil by Bassewitz under the title of "angiofibroma cutis circumscriptum contagiosum," and the similarity from a clinical standpoint between this condition and verruga peruviana has been pointed out on page 136. Histological examination of preparations from the lesions of angiofibroma contagiosum have been made by Unna,[1] by Austrogésilo,[2] by Jeanselme,[3] and by Bennecke.[4] All of these authors emphasize the presence of very numerous young capillaries in the tissue. One cannot fail to be impressed by the similarity from a histological stand-

[1] Unna: Arch. f. Schiffs- u. Tropenhyg., Leipz., 1906, x, 204.
[2] Austrogésilo: ibid., 205.
[3] Jeanselme: Rev. de méd. et d'hyg. trop., Par., 1906, iii, 124.
[4] Bennecke: Arch. f. Schiffs- u. Tropenhyg., Leipz., 1906, x, 297.

point of these lesions and those of verruga peruviana, particularly from the description of Bennecke. Rocha-Lima points out that the processes from the descriptions given do not appear to be identical, chiefly because in the telangiectatic granulomata the formation of vessels, and in the verruga lesion the growth of endothelial cells, appears to be the most characteristic appearance. However, Bennecke emphasizes the granulomatous nature of the angiofibroma contagiosum, the proliferation and new formation of capillaries, and the proliferation of the angioblasts. Whether true fibroblasts were present he was not willing to decide definitely. He suggests that the lesions are of an infectious nature and probably result from a living virus. It therefore seems evident that from a histological standpoint as well as a clinical one the disease angiofibroma cutis circumscriptum contagiosum, if not identical, is probably a very closely allied disease to verruga peruviana. Further observations upon this question are, however, desirable before a definite conclusion regarding their identity can be reached.

Vaccination and Immunity

In Peru the popular opinion prevails that one attack of verruga peruviana confers immunity against subsequent attacks. It is certainly exceptional to find individuals who have suffered two distinct attacks of the disease. We have performed experiments in animals with the idea of discovering whether immunity resulted after inoculation of the verruga virus. We have called attention to the fact elsewhere in this report, that when the monkey is inoculated with the virus either directly from man or from another monkey, no generalized eruption occurs, but just as in the case when the calf is inoculated with small-pox virus, only a localized lesion develops. We have found that monkeys may be very successfully immunized by a single cutaneous inoculation of the verruga virus, and when such monkeys are reinoculated no lesion develops. In all instances in which the monkey is

successfully vaccinated with the virus in this manner, it has been protected against a second attempt at infection. By successful vaccination we imply the development of the local lesion following the inoculation. Animals have been found to be immune for as long a period as six months after the primary inoculation. It therefore seems probable that by using the virus after several passages through monkeys, that man may also be successfully vaccinated against this disease in a similar manner to that which is employed against small-pox. The virus of verruga, however, so far has not been successfully glycerinized, as we have called attention to elsewhere. This method of vaccination against verruga seems favorable since in one human case that was inoculated with the virus only localized lesions developed. It is hoped that in the near future vaccination may be given a careful trial in Peru where the disease is so prevalent.

The discovery of this method of immunization is obviously very important. Indeed it seems likely that the immunity obtained from vaccination against verruga may become as successful as vaccination against small-pox. It also seems probable that vaccination in man may be performed with safety as far as the disease verruga peruviana is concerned, as in small-pox, with virus obtained directly from the human lesions. Experiments of this nature, however, must be pursued cautiously, since as we have pointed out on page 124, material from the lesions in the different stages of the disease seems to possess a different infectivity, as evidenced by its varying power to produce lesions in inoculated animals.

Transmission of Verruga Peruviana by Insects

Upon our arrival in Lima we found the government entomologist, Charles H. T. Townsend, in charge of an investigation by the Peruvian government of the insect transmission of verruga peruviana. This work was begun some time before our arrival, and for several reasons this subject was not directly pursued by us.

In one of Townsend's [1] first articles upon the subject of the insect transmission of verruga peruviana he states: "The Octodontidae and especially the Cricetinae, relatives of the pocket mice and grasshopper mice, are very abundant in forms in the Andean region, and it seems most probable that among them is to be found the primary reservoir of verruga."

"It seems strongly indicated that verruga is transmitted by ticks in practically the same manner as is Rocky Mountain spotted fever — that is to say, that the early stages of the tick live upon the small native mammals, while the adults attach to large animals and man for engorgement during which process they transmit the disease. This explains the mular eruption in mules in the verruga districts."

In March, 1913,[2] he writes regarding the transmission of the disease, "We may therefore confidently exclude fleas, bedbugs, lice, and all other partly or wholly nocturnal bloodsuckers except ticks. The strictly day-biting bloodsuckers — that is to say, those which never bite at night, such as buffalo gnats, sandflies, horseflies, and stable flies — are excluded because it is certain that verruga is contracted oftenest at night. Thus, by a process of exclusion, we arrive at ticks as apparently the only possible carrier of the disease. Most of the other bloodsuckers are excluded, as a matter of fact, because they bear little relation to the burrowing rodent fauna of the verruga districts."

It is notable that he excludes the sandflies as transmitters of the disease. In July, 1913, he changed his ideas regarding the transmission of verruga, and in a third article [3] he writes: "Starting with the indisputable fact of the inoculability of the disease directly into the blood, and the impossibility of contracting it in any other way, we deduce with certainty that it is transmitted by a bloodsucker. The study of the bloodsucking fauna of the Rimac verruga zone, carried on by the writer both by day and by night, has to date disclosed some

[1] Townsend: J. Economic Entomology, Phila., 1913, vi, 211.
[2] Townsend: The Inca Chronicle, Peru, 1913, March, v, No. 3, p. 14.
[3] Townsend: Peru To-day, 1913, July, v, No. 4, p. 840.

fifty species which have this habit. These species may be grouped under the twelve common heads of *mosquitos* (zancudos or culicids), *punkies* (chironomids), *sandflies* (phlebotomids), *buffalognats* (simulids), *horseflies* (tabanids), *stable flies* (stomoxids), *tickflies* (nymphiparids), *fleas, lice, bugs, ticks* and *mites*. All of the above species have been found also outside the verruga zone excepting only the *Phlebotomus*, and several species of buffalognats and horseflies. The last two bite only by day and are therefore at once excluded as verruga carriers, while it is at once evident that all of those which extend outside the verruga zone are likewise excluded. This leaves the Phlebotomus as the only agent present, capable of carrying the disease."

He concludes this article with the statement: "The entomological evidence altogether against the Phlebotomus as the vector of verruga is so strong that it must be considered amply sufficient to convict, even in the absence of the transmissional verification."

In November, 1913, he reported [1] upon "The first case of experimental transmission of verruga accomplished through the medium of *Phlebotomus verrucarum* Townsend." The experiment is described as follows:

"Two hairless dogs of the species *Canis caraibicus* (Lesson), male and female, each about one and one-half years of age, secured in Chosica, April 24, 1913, were admitted to the verruga laboratory on that date, since which time neither one has left the laboratory at any time, the two having been kept chained side by side continuously. The female was used for the transmission, while the male was reserved for the check.

"Up to July 10, 1913, the general condition of the female had been perfect. Examination of her blood made June 15, showed nothing abnormal. The rectal temperature, taken daily since May 3, varied from 37° to 39°.4 C. (98°.6 to 102°.9 F.), with an average of 38°.6 C. (101°.5 F.). She had always been robust, and extraordinarily full of animation.

"On the afternoon of July 11, 1913, I injected the female subcutaneously in the right shoulder with 1 c.c. of artificial serum containing the triturated bodies of twenty females of the *Phlebotomus*, collected on the night of July 9–10 in Verrugas Canyon, none of which showed any sign of blood-meal in the alimentary canal.

[1] Townsend: J. Am. M. Ass., Chicago, 1913, lxi, No. 19, p. 1717.

" The rectal temperatures of this dog taken daily at about 11 A.M. and 5 P.M., were as follows:

	A.M.	P.M.	
July 11	38.6	37.5	(after injection)
" 12	39.1	38.7	
" 13	38.7	38.9	
" 14	38.9	39.1	
" 15	37.7	38.6	
" 16	38.6	38.35	
" 17	37.8	38.5	

" These temperatures indicate nothing abnormal, but are an important part of the clinical history of the case.

" July 16, the dog was noted to be decidedly thin and weak, ribs showing plainly, very little appetite, almost entire lack of animation, and very sensitive to handling. Blood-smear made on that date showed a limited number of endoglobular bodies closely approximating Barton's x-bodies some endoglobular and a large number of exoglobular bodies closely similar to the x-bodies figured by Darling, extremely numerous remains of broken-down red corpuscles and many nucleated reds.

" July 17 there appeared a typical eruption of the nodular type on the superior surface of the right hind foot, the papules being in the incipient stage and not yet developed into subspherical nodules, six of the papules being well covered, while numerous others with little color were present on both the right and left hind feet.

" On the morning of July 18, the six papules showed deeper color. Blood-smear made at the time, from point well removed from the eruption, showed the same very numerous remains of broken-down reds as that of July 16, and a large number of the exoglobular bodies approximating the x-bodies of Darling, but practically no endoglobular x-bodies.

" On the morning of July 19, the dog was still thin in flesh, but appeared much stronger and almost as animated as usual. The eruption was practically the same, except that it appeared less deeply colored. The best colored papule was opened and a smear made from it, which showed, besides some nucleated reds and many remains of broken-down reds, a considerable number of bodies bearing a certain resemblance to *Leishmania*, but lacking the kinetonucleus and manifestly not that organism. I have found the same type of bodies in the *Phlebotomus*, and shall present description and drawings of them later. The exoglobular x-bodies were also present. A smear made at the same time, from a point removed from the eruption, showed remains of broken-down reds, but not in such abundance as July 16 and 18.

" The check dog to date has showed nothing abnormal in either his blood, temperature, skin or general condition, but has remained absolutely the same in all respects. Blood-smear made July 18, showed no broken-down reds and no x-bodies either endoglobular or exoglobular.

"Aside from this being the first experimental demonstration of the insect transmission of verruga, the case is of great interest from a clinical point of view. The practical absence of fever, while unusual, has been noted a good many times; but the remarkably short period elapsing between infection and eruption claims especial attention."

It will be seen from Townsend's account of this experiment that he believes that by inoculating a dog with Phlebotomus flies caught at random in the verruga canyon, he produced the first or febrile stage of verruga peruviana in the animal, found in the blood of this animal the characteristic parasites, and that subsequently a typical eruption of the nodular type appeared upon the right hind foot of the animal. The account of this experiment first appeared as an article signed by Mr. Townsend in one of the daily newspapers during our stay in Peru, and the public was invited to inspect the dog and to examine the lesions described. We accordingly went to Chosica and examined the animal in question. Mr. Townsend was away at the time, but his assistant kindly allowed us to observe the dog and to make microscopical preparations of its blood. The dog appeared active and fairly healthy. There were a few dried pustules measuring about 1 mm. in diameter, and a small grayish indefinite mass of old scar tissue measuring about 4 mm. in diameter on the right hind leg of the dog. The lesions shown us consisted of a few dried-up pustules produced perhaps by staphylococcus, and resembled in no way the lesions of verruga. Several specimens of the dog's blood were taken, and these we stained by Wright's and Giemsa's stains, and examined for parasites. No microörganisms were found therein. Subsequently Mr. Townsend brought specimens of this dog's blood to the laboratory and demonstrated to us the "x-bodies" which he had observed and described in his report. The red blood-cells in these specimens were very badly crenated, the specimens had evidently been improperly hardened, and they showed a very great abundance of artefacts and foreign matter. No bodies resembling the parasite of Oroya fever were observed.

During the present year Townsend [1] has again reported upon the transmission of verruga by insects, and has given an account of an attack of fever suffered by Mr. Nicholson following bites on the back of his hands and wrists. This report might at first sight seem of considerable importance, and it therefore is advisable to refer to it. It appears from the report that Mr. Nicholson was bitten by the flies on the 17th of September. To quote from the report: "Daily examination of Mr. Nicholson's blood revealed nothing abnormal until October 1, when I found what I considered to be the verruga x-bodies in the red cells, but Dr. A. L. Barton, the best known authority on verruga, pronounced them not so. This was due to the smear having been somewhat overstained as compared with Dr. Barton's customary practice in staining. These x-bodies continued in very small number without clinical symptoms of note, other than a headache or slight feverishness at times, until October 25, when a decided rise of temperature occurred and the x-bodies were found to be much increased in number. Dr. Barton now recognizes these to be the verruga x-bodies."

It appears from Townsend's second report that on November 15th the patient's fever "subsided for good." On December 24, the report states the first sign of eruption appeared. On December 28 and January 8, the appearance of further eruption upon the skin is referred to. Thus it would appear from the report that the eruption described did not begin to appear until thirty-nine days after Mr. Nicholson's fever had entirely subsided, and that the eruption did not occur until over three months from the time that he was bitten accidentally through his mosquito net while sleeping in a "Verrugas Canyon."

In another publication in Science Townsend [2] again reiterates his views regarding the disease verruga peruviana. In his most recent publication upon the subject [3] he reports another experiment in which an attempt was made to observe the

[1] Townsend: Entomological News, Phila., 1914, xxv, No. 1, p. 40; No. 3, p. 131.
[2] Townsend: Science, N. Y., 1914, Jan. 16, xxxix, 99.
[3] Townsend: Peru Today, 1914, June, vi, No. 2, p. 57.

development of verruga in a patient bitten by Phlebotomus. The flies were brought alive daily for two weeks from Verrugas Canyon, to the hospital in Callao where the experiment was apparently carried on. "The patient received one hundred and ninety-nine bites, chiefly on the arms, during twenty-five hours and fifty-five minutes total exposure to nine hundred and eighty-one Phlebotomus from February 8 to 23, 1914." He states further: "The temperature rose suddenly February 25, 1914, continuing to rise throughout the following day, but fluctuating thereafter till it reached normal March 1, and continuing only slightly above normal if at all for two weeks. It rose March 14, and fluctuated till the 17th; rose the 20th and 31st of March, the 2d to 5th of April, and again more decidedly the 9th and 10th of April, with a slight rise on the 20th of April as the last sign of the fever. The blood showed leucocytosis, eosinophiles and the sparse presence of bodies which the writer identifies as Bartonia. No sign of malaria was present in the blood, nor other parasite that might have produced the fever. During the last half of March articulation pains were prominent. The patient left for Liverpool June 2, 1914, with letters to Professor Robert Newstead, of the Liverpool School of Tropical Medicine, and Dr. G. H. F. Nuttall, of the University of Cambridge, asking that they examine him for verruga eruption up to August and later if possible, photographing any eruption that may be found."

Apparently no further publication relating to this patient has been made.

Townsend further writes:[1] "Bacilliform bodies indistinguishable morphologically from the Bartonia have been found by the writer in the Phlebotomus, in the lizard blood, in the blood of one guinea pig injected with the lizard blood, in the liver of the other guinea pig similarly injected, in human verruga eruptive tissue; and *not* in rats, burros, dogs, owls, or doves examined in Verrugas Canyon. The first guinea pig injected died in nine days. The second one received a much stronger injection of the lizard blood and died in ten hours.

[1] Townsend: loc. cit.

The bodies seem to be similar to the bacillus, either paratyphoid B or one closely allied to it, which Dr. Barton found present in all of the verruga cases which he studied from 1900 to 1909 and with which he produced both fever and eruption in dogs and mules. All of this indicates the possibility of the Bartonia being only a form of the bacillus modified by parasitism of the ultramicroscopic verruga organism, and that the bacillus carries the actual infective organisms in its substance. At all events the constant presence in verruga blood and tissues of this bacillus, which appears to be the same that occurs so abundantly in the lizard blood, makes it practically certain that it bears a very important relation to the disease." [1] These statements, it would appear, require no comment.

Further experiments will probably show whether a species of Phlebotomus is the transmitting agent in Oroya fever. While we have some confidence in Mr. Townsend's entomological work, we feel from what we saw of his work during our stay in Peru that from a medical standpoint his observations need confirmation before they can be accepted.

It seems very possible that some arthropod is concerned in the transmission of verruga peruviana, but obviously this fact has not as yet been demonstrated, and it is also possible that the disease may be transmitted in a somewhat similar manner to small-pox. A modified form of it may be transmitted by direct inoculation, as has been shown by us. Arce [2] suggested in 1889 that verruga might be transmitted through the agency of some bloodsucking insect, but he referred particularly to the *fiebre grave de Carrion*. Plehn [3] and Castellani [4] also suggest that the cause of the febrile stage may be associated with some bloodsucking animal. As mentioned in Section III, and on page 42, it seems very probable that Oroya fever is transmitted by some arthropod. Whether this is a species of tick, a mosquito, or Phlebotomus, however, has not yet in our opinion been conclusively demonstrated.

[1] Not italicized in the original.

[2] Arce, Julian: Thesis of Lima, 1889.

[3] Plehn: Mense, Handbuch der Tropenkrankheiten, Leipz., 1905, 1st ed., ii, 439.

[4] Castellani: Castellani and Chalmers, Manual of Tropical Medicine, Lond., 1913, 2d ed., 1200.

V

ENTOMOLOGICAL INVESTIGATIONS AT MATUCANA

Entomological investigations were made in Peru, particularly at Matucana, Surco, and San Bartolomé, three towns in the " verruga zone " along the Rio Rimac. These towns are all stations on the railroad which extends from Callao and Lima into the mountains to Oroya. Verruga peruviana, Oroya fever, and malaria occur at all three places; Matucana with an elevation of 7,300 feet being near the upper limit of the " verruga zone," while San Bartolomé with an altitude of about 4,000 feet is not very far above its lower limit.

The physiography, climate, and the flora and fauna of the country about and between these towns, especially the former, have often been discussed by Odriozola and others, and have been referred to on page 58.

During the week spent at Matucana, late in June, the temperature rose daily to about 74° F. in the shade, and was much higher in the sunny parts of the canyon. During this part of the day there was a strong breeze blowing upwards. After the sun had set behind the tops of the mountains, the temperature rapidly fell often to below 50° F. at night, at which time the direction of the wind seemed regularly to be reversed. There was thus a great diurnal range in temperature, from the hot day to the chilly or even cold night. In the sheltered portions of the canyon, especially along some of the streams that flowed down the lateral canyons, the temperature did not rise so high owing to the failure of the sun's rays to penetrate into these places. These smaller side canyons have always been associated with verruga and Oroya fever, particularly the notorious " verrugas canyon " where these diseases appeared so abundantly during the construction of the railway bridge at this point. (Plate xviii, Fig. 3, page 59.)

The insect fauna is by no means meager at Matucana in spite of the extremely dry environment. Beneath the stones in the exposed places were numerous Carabid and Tenebrionid beetles and a species of scorpion, *Hadruroides lunatus* Koch is quite abundant. Most conspicuous along the trail are various bees, of which a number of species were taken. These have been kindly examined by Professor Cockerell who has already published on them [1] and a list of the species is included in the Appendix (p. 220).

Diptera were well represented, although the smaller forms, like the acalyptrate Muscidae which are usually associated with more humid conditions were scarce except about the water which is diverted from the river. A few Tabanidae were collected. Among these were two large species probably of the genus Pangonia, noticeable on account of the long, porrect proboscis. They were seen visiting the flowers of a Mimosa-like shrub, but also hovered about us as if seeking an opportunity to bite. The guide stated that these flies regularly bite persons, but an examination of the alimentary tract disclosed no blood in any of the specimens captured. The other species, belonging to the genus Tabanus, were taken along the trail, also seeking an opportunity to bite as is usual with members of this genus. No blood-sucking Muscidae were seen at Matucana, although *Stomoxys calcitrans* was seen at San Bartolomé. It is far more common, however, at lower altitudes, particularly in Lima and Callao.

Mosquitoes were very scarce, but one species was reared in large numbers from larvae found in the stagnant water that filled two large concrete pits close to Matucana. These tanks are used as a supply for the railroad water tank at the Matucana railroad station and were teeming with larvae. Specimens reared from these have been examined by Dr. H. S. Dyar and Mr. F. Knab of the United States National Museum, who find them to represent a new species of an hitherto undescribed genus. This species therefore is of considerable interest, particularly as we are able to give a description of the larva and pupa.

[1] J. N. Y. Entom. Soc., 1914, xxii, pp. 306–328.

Phalangomyia Dyar and Knab. Adult

" Proboscis long, uniform, nearly straight in both sexes, in the male with a false joint near the middle. Palpi short in the female; in the male long and slender, acuminate. Antennae filiform in the female, the joints with basal whorls of short sparse hairs; in the male sparsely plumose, rather long. Clypeus well developed, nude.

" Prothoracic lobes small, lateral. Mesonotum elongate, convex, with longitudinal series of coarse hairs on the disk and laterally. Scutellum weakly trilobe. Pronotum convex, prominent, nude.

" Abdomen subcylindrical in the female, truncate at tip; in the male long, slender basally, depressed outwardly.

" Legs slender throughout and very long, the three pairs progressively longer but not differing greatly in length; femora and tibiae of all three pairs of legs of about equal length; first joint of hind tarsi slightly shorter than the tibia; scraper of hind tibia without complete row of spines. Claws simple and small in the female; in the male large, unequal, and each with a single tooth on the front and middle legs.

" Wings with the venation as in Culex.

Phalangomyia debilis Dyar and Knab

" Female: Proboscis clothed with dark brownish scales. Palpi about one-sixth as long as the proboscis, dark scaled, with a few coarse bristles. Clypeus prominent, narrow, rounded anteriorly, dark brown. Antennae rather long and slender, blackish, with white rings at bases of joints; tori small, yellowish. Occiput blackish, clothed with narrow curved yellowish scales, broader and denser white ones along ocular margins, many erect, very slender, forked brown scales dorsally; cheeks white scaled.

" Mesonotum dark brown, two bare, narrow, longitudinal lines, the antescutellar space bare, a large bare area laterally on posterior half of disk; scales rather sparse but coarse, narrow curved, yellow-brown, larger pale scales along margins and on posterior half about the three bare zones. Scutellum with pale scales, like those preceding, each lobe with a large group of coarse black bristles. Pleurae brown, with lanceolate white scales.

" Abdomen rather slender, depressed dorsally; dorsal vestiture dull black, the segments with very broad, basal yellowish white bands, the band on the second segment medianly produced to posterior margin, the white on the sixth and seventh segments occupying more than the basal halves, the eighth entirely white scaled; no differentiated lateral spots; venter uniformly dirty white scaled; many long, pale yellowish bristles at posterior margins of segments and ventrally, in appearance approaching the lateral ciliation present in many male mosquitoes.

" Legs brownish black, the tibiae and tarsi with pale luster beneath; femora pale beneath to near apices; knees pale; tibiae narrowly pale at bases, the tips of all three pairs broadly white; tarsi unbanded. Claw formula, 0.0–0.0–0.0.

" Wings hyaline, moderately broad; second marginal cell rather narrow, nearly three times as long as its petiole, second posterior cell slightly longer than its petiole; scales dark brown, paler, yellowish, at tip of wing in a spot involving tip of first vein and apical halves of forks of second vein; outstanding scales long and linear, the others not differentiated; fringe narrow, unicolorous. Halteres dark, scaled throughout.

" Length: Body about 5 mm., wing 6 mm.

" Male: Palpi slender, nearly uniform, upcurved, exceeding the proboscis by about the length of the last joint; vestiture brownish, without pale rings, rather sparse, short, stiff black hairs at end of long joint and along last two joints. Antennae rather long; last two joints long, the others shorter, slender, pale, with narrow black rings at insertions of hairwhorls; hairs very long, moderately abundant, brown, shining; tori large, luteous. Coloration similar to the female. Abdomen long, slender near base, depressed beyond; white dorsal bands broader than in the female, occupying about two-thirds of sixth and seventh segments, eighth wholly white scaled; lateral ciliation long and fine, pale yellowish. Wings nearly as broad as in the female; stems of fork-cells but slightly longer. Claw formula, 1.1–1.1–0.0.

" Length: Body about 4 mm., wing 4 mm.

" Matucana, Peru, 7,300 feet, June–July, 1913 (C. T. Brues).

Types and paratypes in the collections of the United States National Museum and of Department of Entomology, Harvard School of Tropical Medicine; one pair deposited in the British Museum. Type, No. 18361, United States Nat. Mus.

" Male genitalia: Side pieces from above broadly conical, straight within, convex without, about twice as long as the greatest diameter, sparsely covered with short hairs, a few long ones on the outer side and a row of about seven long ones closely crowded in a line at the tip before the insertion of the clasp filament. Beneath the inner area of the piece is cut away to two-thirds of its base, but not any at the tip. The remainder of the piece forms a ridge, elongate, about three times as long as wide, deeply excavate on the inner side in the central third, regularly convex without, the tip shortly truncate at the insertion of the clasp filament; the inner angle basally of the emargination is densely hairy, with distinct tubercles; beyond the emargination is a large dark chitinous cone, from the outer aspect of which arise (1) a heavy brown rod which expands beyond the middle into a half-disk and ends in a digitate point, and (2) a long thin leaf-like appendage, hardly widening outwardly, truncate at tip, its inner and distal edges deeply dentate, spinose, its outer angle prolonged into a long point. Within the emargination of the under side of the side piece is a broadly triangular lobe, its outer angle fitting into and surpassing the emargination, dark and densely tubercular and bearing a brush of densely crowded setae, especially long and dense at the angle. This triangular lobe is joined by a membrane to a stout chitinous strip on the inner side, at the termination of which are two spine-like rods, inserted in tubercles, with pointed, slightly recurved tips. Clasp filament stout, enlarged a little at the base, bent beyond the middle, where it becomes a little inflated and creased; tip slender, furcate, with two widely divergent points. Harpes broad, triangularly rounded, the inner angle blunt and bearing a dense crown of short spines; outer angle forming a slender recurved spiral. Harpagones small, plate-like, single, bearing three or four stout curved teeth toward the tip. Unci small, narrow, with rounded tips. No basal appendages.

" The genitalia are plainly of the Culex type, as shown by the presence of the prominence bearing a leaf-like appendage and the tufted harpes. The side pieces are specialized and curiously elaborated, but the undivided harpagones and the presence of the lobe indicate a low origin for this form. The lobe has disappeared from all the Culex proper, being seen only in low forms which are not truly Culex, such as *C. dyari*, *C. melanurus*, and the species of Culiseta. In these the characteristic structures of Culex have not appeared; but they are well shown in Phalangomyia."

" This genus, therefore, forms a connecting link between Culiseta and Culex, nearest to the latter and modified on its own peculiar lines."

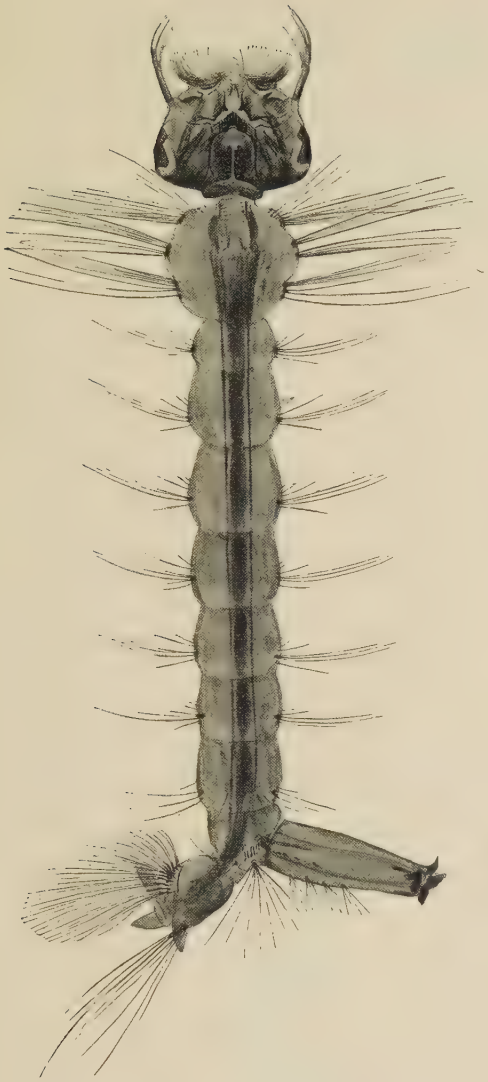


Fig. 1. — Phalangomyia debilis Dyar and Knab.
Full-grown larva, dorsal view.

Fig. 2. — Phalangomyia debilis Dyar and Knab. Pupa, lateral view.

Fig. 3. — Aphiochaeta scalaris Lw.
Lateral view of male.

Plate XXXIII.

A description of the larva and a few sketches illustrating the more important anatomical details of this mosquito follow. (Plate XXXIII, Fig. 1.)

Phalangomyia debilis Dyar & Knab

Larva. Length of full-grown specimens 8 to 9 mm. Head subtriangular, about as broad as long, with rounded anterior margin and rather strongly projecting

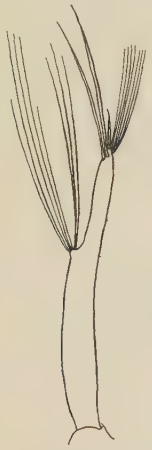


FIG. 1. — *Phalangomyia debilis*, Antenna of larva.

FIG. 2. — *Phalangomyia debilis*, Mandible of larva.

angulations for the insertion of the antennae. Antennae (Fig. 1 Text) with the lateral tuft beyond the middle, just before the apical third. Mouth brush extensive

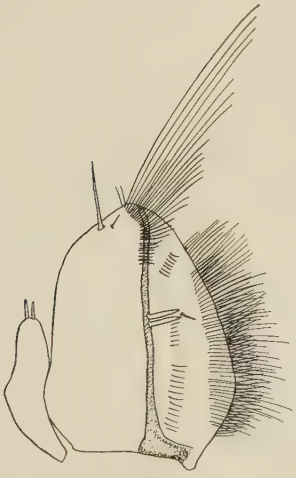


FIG. 3. — *Phalangomyia debilis*, Maxilla.

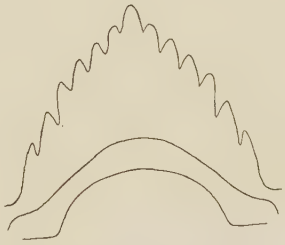


FIG. 4. — *Phalangomyia debilis*, Mental plate of larva.

and rather diffuse, most of the bristles usually extending out laterally and curved forward in preserved specimens. Mandible (Fig. 2 Text) with two stout setae

above the middle of the external surface just preceding the apical fan; apex with five teeth of nearly the same size. Maxilla (Fig. 3 Text) subovate, one-half longer than broad, with one apical seta, its small lobe with two short setae: apical tuft long, consisting of delicate bristles. Mental plate (Fig. 4 Text) with six teeth on each side below the apical tooth. Head above with a transverse series of tufts of bristles across the middle, well behind the base of the antennae. Lateral bristles of thorax not extending on to the disc except for several very sparse tufts about midway between the anterior and posterior margins. Abdominal bristle tufts on the lateral margin moderately developed; first and second segments each with two tufts of four larger and two smaller bristles respectively, third, fourth, and fifth each with a single tuft of four; six and seventh each with two or three. In addition

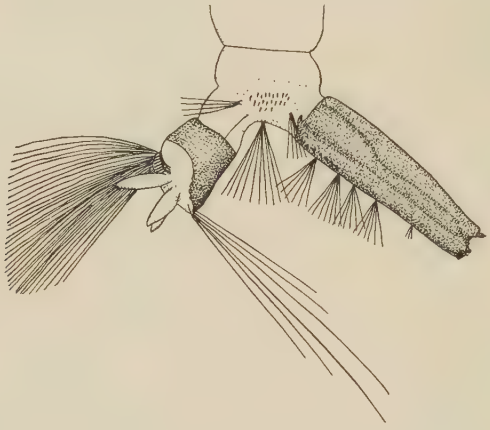


Fig. 5. — *Phalangomyia debilis.* Posterior portion of larva.

there are on all the segments a few minute hairs or bristles. Seventh segment without a dorsal or lateral plate. Lateral comb of the eighth segment consisting of a transversely oval group of spine-like scales about 25 to 30 in number and not arranged in distinct rows. Air-tube (Fig 5 Text) almost three and one-half times as long as broad at the widest place near the base, slightly and gradually tapering, not inflated nor swollen; its pecten consisting of about seven very short spines; its hairs placed in four tufts with sometimes a minute fifth one in addition. Anal segment with the chitinous ring much narrowed ventrally, only half as broad there as above; anal processes broadly lanceolate, projecting usually about half the width of the anal ring.

Described from many specimens taken at Matucana, Peru, 7,300 feet, in a concrete pit containing water used as a supply for the railroad locomotives.

From a very large lot, all alike, only specimens of *P. debilis* were reared, so that there is no possibility of mistake in associating the larvae and adults.

The larva appears in most of its characters to be closest to Aëdes, with which it agrees in the form of the respiratory

siphon, and the short spine-like bristles of the pecten on this organ. The ventral narrowing of the ring on the anal segment and the more numerous tufts of hairs on the siphon are similar to Culex. From Culiseta it differs entirely in the armature of the siphon. The dentition of the mental plate might be that of certain species of either Culex or Aëdes.

The pupa (Plate XXXIII, Fig. 2) does not show any striking peculiarities.

Larvae of Simulium (Plate XXXIV, Fig. 2) are abundant in the vicinity of Matucana, and a number were collected in a small mountain stream that enters the Rio Rimac just below the town. The season for adults was evidently just approaching as none were seen beneath the bridge which crosses at this point nor in the surrounding region. These larvae evidently belong to two species as is shown by the quite different conformation of the labium and other structures. With the larvae were taken two very similar, but distinguishable types of pupae in addition to two others of widely different appearance. These latter were enclosed in a cocoon of the usual Simulium type, but are each provided with such a peculiarly modified type of pro-thoracic respiratory apparatus, that they have been referred to this family with some doubt as apparently no similar organs have been described either in this or related families.

As the larvae and pupae furnish very good characters, the forms obtained have been described without however giving them names in the absence of adult specimens. A number of species of Simulium are known from Peru [1] and quite likely the adults of some of the forms here described have already been named.

Larva No. 1

Length 7 to 9 mm. The fans have about sixty or sixty-five rays. Antennae with the second joint three times as long as thick at the base, one half as long as the first, first and second joints pale brown, second hyaline at base and apex; third darker brown. The mandibles possess the apical pair of external bristles; the large apical teeth and the two following are black, the remainder of the smaller teeth light brown. Maxillary palpus without bristles, black, the apex hyaline. Labium (Fig. 6 Text) with the dentate apical margin rather narrow, the median tooth simple, scarcely projecting beyond a line connecting the apices of the lateral teeth;

[1] Knab, F.: Simuliidae of Peru. Proc. Biol. Soc., Wash., 1914, xxvii, pp. 81–85.

between the median and lateral tooth are three small teeth, the lateral one of these larger than the others; along the side of the labium is an oblique close-set row of seven bristles, followed by a smaller pair and some distance behind another smaller one, making ten in all. The head is very dark, piceous in fully colored individuals; the thorax dark gray except for the region about the ventral proleg and the abdo-

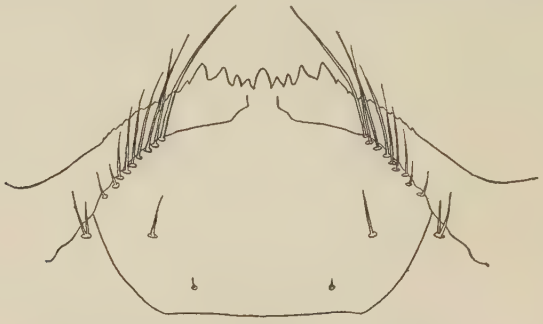


Fig. 6. — Simulium larva, No. 1. Labium.

men is gray except below on the apical half. The anal respiratory organ is trilobed, the three lobes usually simple, sometimes the lateral ones each with a small thumb-shaped secondary lobe at the base; rarely with the median one also thus divided. In at least some individuals with simple gill-lobes there are only five bristles on the side of the labium, indicating perhaps a varietal form, but as a gill lobe may sometimes be present on one side and not on the other the two forms are not very distinct.

This was the most common form among the larvae obtained.

Larva No. 2

Length 10 mm. The fans have from twenty-five to thirty rays. Antennae with the second joint about four times as long as thick and half as long as the first; all three joints pale yellow. Mandibles with the large apical teeth black, the others pale. Maxillary palpi black, not bristly, apex pale. Labium (Fig. 7 Text) with

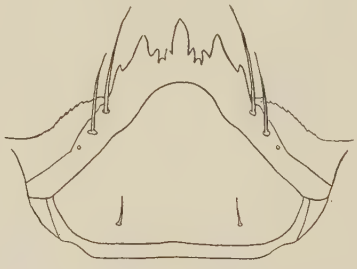


Fig. 7. — Simulium larva, No. 2. Labium.

the dentate apical margin narrow; median tooth large, trifid as the adjacent small tooth is advanced forward, next tooth very small, next larger, followed by a large lateral tooth which is shorter than the median one; along the side of the labium is a row of three bristles, the anterior one large, the other two growing successively smaller. The anal respiratory organs are not extended in any of the specimens and I have been unable either to evert or observe them in examples treated with potash or peroxide, although the integument covering these organs is not destroyed by such treatment.

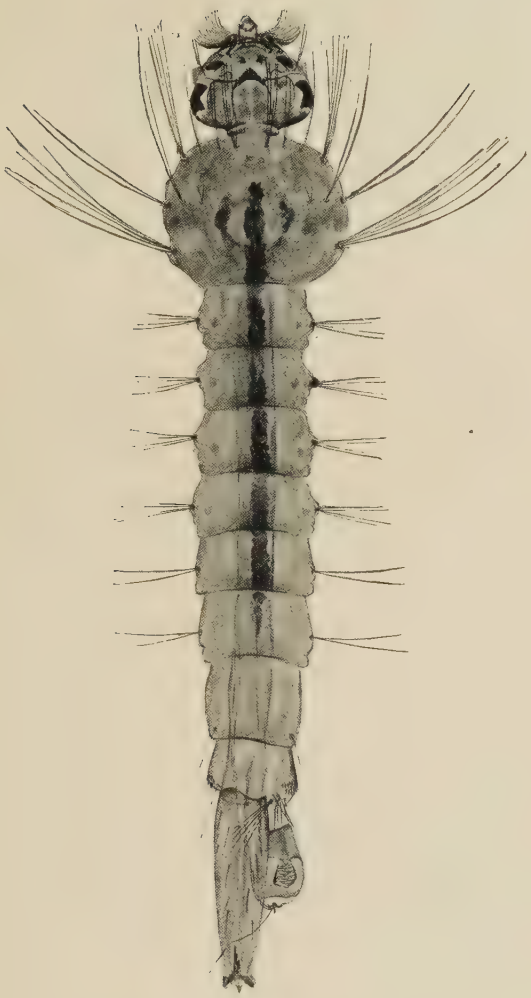


Fig. 1. — Culicine Larva.
Full-grown, dorsal view. Surco.

Fig. 2. — Simulium sp.
Full-grown larva. Matucana.

Plate XXXIV.

Pupa No. 1

Length 3.8 to 4.2 mm. Body entirely enclosed in the cocoon which projects as a rim above the slightly oblique, truncate anterior surface of the head. Four respiratory filaments on each side, the main trunk dividing into two branches very near its base; each of these then divides almost immediately, forming the four filaments which do not subdivide again before the apex except rarely in an occasional filament which divides once near its tip. Arising from the main trunk at its extreme base there are in addition two shorter filaments which extend backwards and lie on the side of the body inside the cocoon. The third and fourth segments of the abdomen each bear dorsally a series of four small hooks on each side ventrally the fifth segment bears on each side of the middle a pair of approximate hooks; the sixth and seventh each bear the same number of hooks, but here the hooks are equidistant from one another, the pair on each side not being approximated.

Pupa No. 2

Length 3.8 to 4.2 mm. Very similar to the preceding form and perhaps only a variation of it. The respiratory trunk divides into two branches at the base, one of these branches again divides a very short distance beyond and the other at double the distance from the base; of these four filaments the dorsal one then later divides into two, the next remains simple. There are thus formed six twigs to the respiratory organ.

One specimen of this form was taken at Matucana, Peru, at an altitude of 7,300 feet.

Pupa No. 3

Length 4 mm. Of an entirely different type from any described pupae of Simuliidae so far as it has been possible to ascertain. Pupa case not attaining the

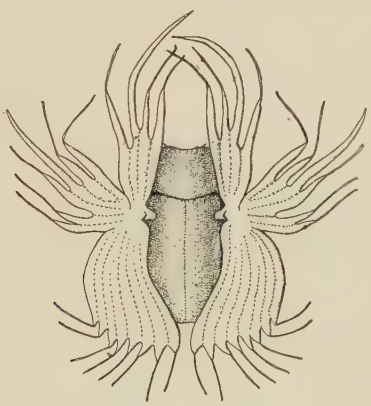


Fig. 8. — Simulium pupa, No. 3. Anterior aspect of the body.

anterior margin of the head, more or less slipper-shaped, but rather loosely woven. Respiratory organs of very peculiar form, arising in the usual position, but spreading out laterally like a ragged palmetto leaf and lying rather flat, in a plane perpendicular to the axis of the body. Viewed from the front the median margin is nearly straight while the lateral margin though very irregular is more or less semicircular. Each fan is composed of eighteen rays connected basally, but separate apically, at

two places the clefts between the rays are much deeper, dividing the rays into three groups of four, six and eight rays respectively; the rays are of nearly the same length, except that the third ray of the first group of four and the third ray of the second group of six are much longer; each ray narrows gradually from the point that it separates from the adjacent ones till it becomes extremely thin, from then the tip is prolonged as a long delicate filament; although the rays are continuous basally, each is sharply marked off from its neighbor by an impressed, punctate line. Figure 8 Text shows the form and position of the respiratory organs as seen from directly in front of the head, the eight-rayed section being dorsal in position. Dorsally the third and fourth segments of the abdomen bear on each side a rather approximated series of four hooklets along the posterior edge; the seventh bears on the edge a continuous comb-like series of very many tiny hooklets and the eighth four equidistant small spines. At the apex of the abdomen is a pair of stout, upwardly directed claws or hooks. The ventral segments bear two small hooks on each side of the posterior margin of the fourth and fifth segments, and one on each side of the margin.

One specimen, collected at Matucana, Peru, at an altitude of 7,300 feet.

Concerning the affinities of this peculiar pupa there appears to be much doubt, but it must undoubtedly belong to the genus Simulium or to some hitherto unknown genus. Aside from the extraordinary respiratory organs, the cocoon, the form of the body and the armature of the abdomen are typically Simulium-like. It is to be hoped that in the near future some one may be able to rear the adult.

Pupa No. 4

Length 5.5 mm. Pupa of still another type with the respiratory organs in the form of balloon-shaped sacs (Fig. 9 Text), one at each side of the thorax in the

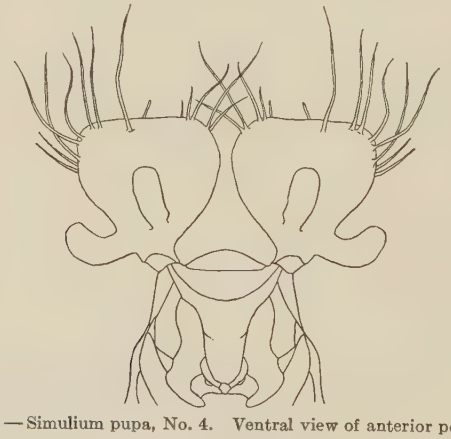


Fig. 9. — Simulium pupa, No. 4. Ventral view of anterior portion.

usual position. Viewed from the ventral side, these are subtriangular in form, each somewhat broader than the anterior margin of the body and narrowed to a very

short, circular pedicel at the point of attachment to the body. Near the base each bears two much smaller polliciform projections, one lateral and the other ventral; about the apex is a crown of slender filaments, similar to the twigs of the usual respiratory organ, which marks the large, convex, more or less circular apical surface. These are of variable length, and very rarely branched. The third and fourth dorsal abdominal segments each bear along the posterior edge the usual series of four hooklets on each side of the middle; the sixth and seventh bear along the posterior edge a comb-like series of close-set tiny hooklets, which become obsolete laterally. The apex of the abdomen bears two upturned hooks; on the ventral side the fourth segment bears a tiny hooklet each side of the middle, the fifth two on each side, the sixth two, and the seventh one.

One specimen, taken from Matucana, Peru, at an altitude of 7,300 feet.

Were it not for the unusual respiratory organs of the preceding species, this one could not have been definitely classified as a Simuliid. It has, however, the other characters of the group. The cocoon, abdominal hooks and the microscopical structure of the respiratory filaments are like that of the " twigs " in pupae of the usual type.

Hypotheses regarding insect transmission. — Several hypotheses have been advanced concerning possible carriers of Oroya fever (usually considered together with verruga under the name of the latter disease), but so far none of these has received actual proof of its accuracy. These are very interesting in the absence of positive evidence and it may be of value to review them briefly.

The character of the organism causing Oroya fever suggests the possibility that some tick may act as a carrier, since Babesia, Theileria, Nicollia, Nuttalia and Smithia as well as Anaplasma and Lympho-haematocytozoa are, so far as known, all transmitted by ticks. However, no species of tick having habits compatible with the distribution and epidemiology of the disease has been discovered. The suggested similarity of Oroya fever to the Rocky Mountain spotted fever of North America, shown conclusively to be tick-borne, is also suggestive of a tick as a carrier for the Peruvian malady. In view of lack of knowledge concerning the organism of spotted fever the analogy is far from complete, however.

Among blood-sucking Diptera there are several families which might contain species fulfilling the requirements of the epidemiology of Oroya fever.

Diligent search about Matucana failed to unearth a single tick of any sort and these animals are evidently by no means of common occurrence. A native guide stated that many animals were infested with ticks and he secured a number of these so-called *carrapatos* which proved to be nothing more than the pupiparous fly, *Melophagus ovinus* which is a common parasite of sheep there as well as northward in Ecuador. No ticks could be secured from horses, cattle and llamas, which are said sometimes to be infested, so that it is impossible to say whether the parasites of these animals are true ticks or Hippoboscid flies.

It is said on good authority that there are no mammals restricted in their distribution to the verruga zones although the viscacha (*Lagidium peruarum*) is typical of these regions from which it extends, however, to much greater altitudes. On this animal, Townsend [1] has collected a Gamasid mite (?*Leiognathus*) and a tick (*Ixodes lagotis*) not only on animals from the verruga zone but also on those much above it. Certain mice may be restricted to the verruga zones, but probably none without very close relatives in the adjacent regions.

Townsend has claimed that the disease is transmitted by a species of Phlebotomus. However, evidence is rather against such a supposition, since as mentioned above the group of organisms to which Bartonella is related appears to be characteristically parasitic in the tick. Nevertheless there are undoubtedly a number of phlebotomic Diptera restricted to the verruga zones. We secured a mosquito at Matucana belonging to an undescribed genus (*Phalangomyia*) which is quite possibly limited to the Oroya fever zones. Townsend has also obtained a species of Phlebotomus at San Bartolomé and Verrugas Canyon which appears not to extend beyond the altitudinal limits of the zones. Simulium is abundant in the Oroya fever zone of the Rio Rimac from whence adults have

[1] Townsend: Bull. Entom. Research, 1913, iv, 125.

been taken as well as the peculiar pupae collected at Matucana and described on another page (167) of this report. Simulium, of course, ordinarily feeds by day as well as by night and also occurs just as commonly in other regions. Whether the adult Simuliidae represented by the highly modified pupae have different habits, must at present remain an open question.

The Phlebotomus discovered and named by Townsend *P. verrucarum* [1] was thought by him to be the carrier of verruga (including Oroya fever). In the first place these insects appear only at night, remaining concealed in the moist herbage and close to the damp rocks of the side canyons during the day. In distribution they are so far as has been ascertained restricted to the verruga zone, in fact conditions suitable to their development certainly do not occur below and probably not above the zone. Thus the distribution of Phlebotomus as far as we know agrees well with the geographical range of both verruga and Oroya fever and its nocturnal activity coincide with the fact that at least Oroya fever is contracted only at night. We have referred to the fact on page 154 that Townsend has reported that a patient contracted Oroya fever after having been bitten accidentally in " Verrugas Canyon " by Phlebotomus and that experiments were then made upon dogs by inoculation with triturated Phlebotomus, which were claimed to have later developed verruga peruviana. These experiments are not convincing as we have shown.

Moreover it will be seen that such a view might be correct only on the assumption that verruga and Oroya fever are one and the same disease, or that Phlebotomus is the carrier of both diseases which obviously hardly seems probable. The implication of Phlebotomus must therefore be regarded as we have already intimated only as a possibility requiring clear cut experimental results for its demonstration. Nevertheless this insect deserves further study, in connection with which it must naturally be borne in mind that there are undoubtedly many other insects in these zones which remain to be discovered, *e. g.*, various mosquitoes (Plate XXXIV, Fig. 1). In

[1] Townsend: Insec. Inscit. Menstruus, 1913, i, 107.

any case the carrier would seem to be a species of nocturnal habits, for it seems probable that the disease is contracted after sunset.

At the same time as emphasized elsewhere in this report (page 160) while Oroya fever can hardly be other than insect- or tick-borne, the evidence for a similar method of transmission for verruga is far less complete and rests entirely on epidemiological considerations. While these are very suggestive, they are by no means conclusive.

FIG. 1. — Early papule above right lip. Large numbers of Leishmania found.

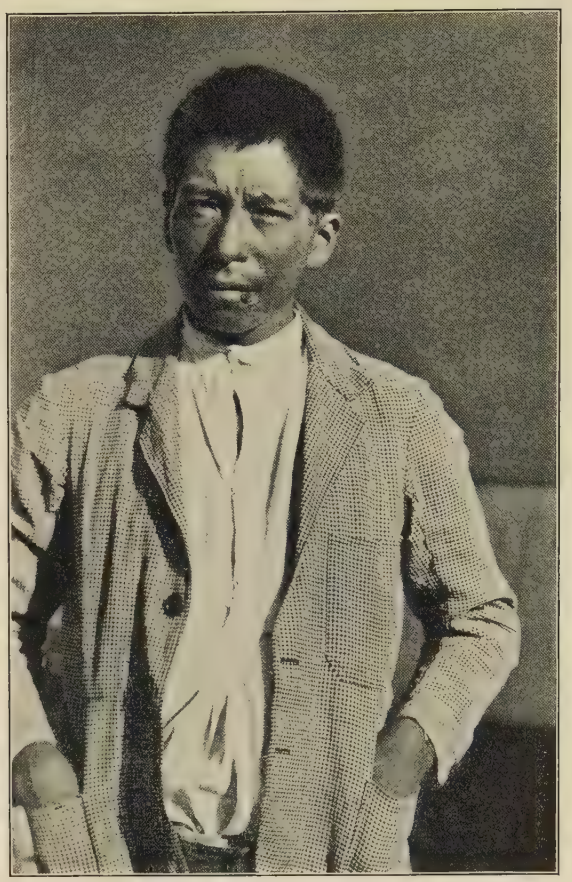


FIG. 2. — Slightly more advanced lesion on lip than No. 1.

PLATE XXXV. — UTA.

Fig. 1. — Early lesions on mother and child, slightly more advanced than shown in Plate xxxv, Fig. 2.

Fig. 2. — Very advanced lesions.

Plate XXXVI. — Uta.

VI

UTA

Uta is a disease which has existed in Peru since prehistoric times, the lesions of which have been supposedly depicted on the ancient "huacos," or pottery, of the Incas. It has been stated in the earlier literature that the disease represented a form of syphilis, or of prehistoric leprosy, or a special form of lupus vulgaris. Smith [1] and Tschudi [2] refer to the malady and point out that it consists of an inflammation of the skin, endemic in various parts of Peru, particularly on the road from Lima to the mines of Cerro de Pasco. According to them the disease consists of an inflammation caused by the boring of a parasite through the skin, frequently of the scrotum, the result being the production of an ulcer which later takes on a cancerous or lupous character. The malady sometimes ends fatally. Hirsch [3] states that uta possibly belongs in the group of diseases caused by larvae of Oestrus, developing in the subcutaneous tissue. In recent years two extensive monographs on the subject have appeared from Peru, one by Tamayo [4] in 1908, and the other by Palma [5] in 1909. Tamayo calls attention to the analogy both from a clinical and histological standpoint between the disease and lupus vulgaris. He however does not believe in the complete identity of the two affections, and thinks that a final opinion regarding this question should be reserved. His monograph is accompanied by numerous photographs illustrating the lesions of the malady as depicted on the water jars of the Incas, and also contains illustrations of the early and late stages of the disease as it is observed today. Palma concluded that it was a specific

[1] Smith: Edinb. M. & S. J., 1840, 339.

[2] Tschudi: Oesterr. med. Wchnschr., Wien, 1846, 509.

[3] Hirsch: Handbook of Geographical and Historical Pathology, Lond., 1885, ii, 370.

[4] Tamayo: La Uta en el Perú, Lima, 1908, 1; Proc. IV the Pan-American Congress, Santiago de Chile, Dec., 1908.

[5] Palma: Boletin del Ministerio de Fomento, Lima, 1908, vi, No. 10, p. 1.

affection which was not to be confounded with other South American maladies, and that it was not a form of tuberculosis. His report also contains many interesting photographs illustrating various lesions of the disease. Anderson,[1] who has observed uta in Peru, compares it with gangosa and states that there are many points of resemblance between the two. He shows that in the absence of syphilis, tuberculosis, and leprosy, the two diseases resemble each other, but that gangosa generally affects the mucous membrane of the throat and pharynx first, in the shape of a nodule, which eventually ulcerates, the ulcer eating through cartilage and bone and finally invading the cheeks and lips. Uta, on the contrary, rarely begins anywhere else than on the skin, not on the mucous membrane, its invasion of the interior of the nose and mouth being only a secondary process, when eventually the upper lip and the nose with its cartilage may be eaten away. The borders of the affected part do not form a ridge, but gradually merge into healthy skin. There is a bright red zone which fades off into the normal color. A crust forms on the ulcerating nodule and in some cases it is connected with epidermic processes which penetrate to the deeper layers of the cutis, constituting what the natives call the "roots" or "feet" of the uta.

The disease was observed by Past-Assistant Surgeon Perry, United States Public Health Service, in his travels through Peru; and by Surgeon General Rupert Blue who, in conversation with one of us prior to our departure from the United States, mentioned its occurrence among the school children of Surco, one of the Peruvian mountain towns in which the disease is very prevalent. In both Surco, Plate XLIV, Fig. 2, page 185, and Otao (the latter town deriving its name from the prevalence of the malady there) a large proportion of the inhabitants are either afflicted with the affection, or show the disfiguring scars which have resulted from a previous attack, on the face, arms, or legs.

[1] Anderson: Proc. xviith Internat. Cong. Med., Lond., 1913, Sect. 21, Trop. Med. & Hyg., 309.

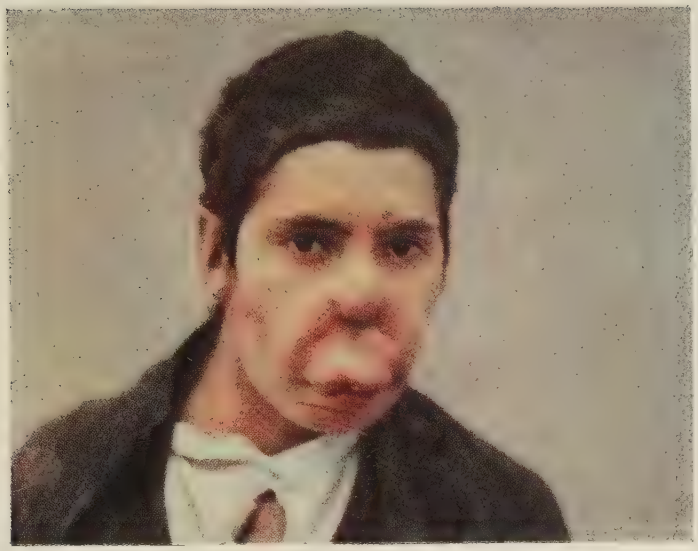


Plate XXXVII. — Uta (From Lumiere Plates).

Healing Stage of an Advanced Case.

The disease begins with a small, insignificant-appearing papule (Plate xxxv) which gradually increases in size, and after a month or two a lesion measuring usually from 1 to 3 cm. in diameter is formed. In this stage of the disease the lesion is covered by a more or less moist, dark crust from which a sticky secretion exudes. On removal of this crust a moist, superficial ulcer which bleeds freely is revealed. In neglected cases the ulcerations extend slowly in size and in depth, and the lesion usually becomes secondarily infected with bacteria and sometimes with spirochaetae. Larvae of *Chrysomyia macellaria* then sometimes penetrate into the depths of the lesions, particularly when they occur in the region of the nose and mouth and pharynx. In such cases the ulcerations become very extensive. The soft and hard palate may be destroyed by the extension of the lesion, and the walls of the pharynx eaten away. Plate xxxvi, Fig. 2 illustrates a case of this nature, specimens from which were obtained through the assistance of Dr. W. F. Bailey. In the great majority of cases which are treated the ulcerations do not penetrate to great depth, and when the infective agent is destroyed the ulcer heals by granulation and a scar results. (Plate xxxvii.) The lesions may be single or multiple; the face, mouth, and lips, ears and neck are more commonly affected, but the ulcerations may occur on the arms or legs. (Plate xxxvi, Fig. 1.) Uta is particularly common in children. Often in the early stages of the disease not a single papule is found, but a group of them occupying an area of 3 or 4 cm. in diameter. The ulcers when they have developed do not have a punched-out appearance but their borders are uneven and ragged. There is usually no marked resemblance between the lesions of uta and those of lupus. Infection can evidently occur by direct inoculation, and we usually found when one child of a family was infected the others also either showed the lesions or the resulting scars. Plate xxxvi, Fig. 1 illustrates a mother and child infected with the disease. Whether transmission also occurs indirectly through the bite of some insect, as seems probable and as is popularly supposed, we are unable definitely to state.

From the study of microscopical preparations made from the lesions of uta and sections of them we were able to show that the disease is due to a species of Leishmania. Drawings made from two of the preparations, and a photomicrograph of a section from one of the lesions are illustrated in Plates XXXVIII to XL. The parasites are found to be very abundant in the early lesions, but they become increasingly more difficult to find as the lesions increase in size. Particularly is this so after secondary infections have occurred. The parasites are found sometimes free, but more often included within the endothelial phagocytes. (Plate XXXVIII.) There is nothing otherwise particularly characteristic regarding the histological appearance of the lesions. They resemble in the earlier stages granulation tissue in which a large number of endothelial and plasma-cells are present. In cultures made upon blood agar from the lesions the flagellate stage of the organism was obtained and dividing forms observed. (Plate XXXIX, Fig. 2.) As the illustration shows, a basal granule in addition to the blepharoplast is present.

The classification of the species of Leishmania is difficult. Laveran and Nattan-Larrier[1] have created the variety *Leishmania tropica* (Wright) var. *americana* and Vianna the variety *Leishmania brasiliensis*. The former was said to be characterized particularly by the flattened nucleus. For the present at least from the evidence available we do not feel justified in creating a new species for the parasite discovered by us as the etiological factor of uta. Attention has been called to the presence of the additional basal granule in the flagellate stage of the parasite of uta. A dog was inoculated on August 1 upon the inner surface of both ears by a stab and scarification with fresh material from a lesion upon the face of a case of uta. On September 16 upon the internal surface of the left ear two papules measuring 3 and 4 mm. in diameter were observed, elevating the surface of the skin. (Plate XL, Fig. 1.) These were whitish in the center and slightly pink at the periphery. In the right ear four similar lesions were also

[1] Laveran and Nattan-Larrier: Bull. Soc. de path. exotique, Par., 1912, v, 176, 486.

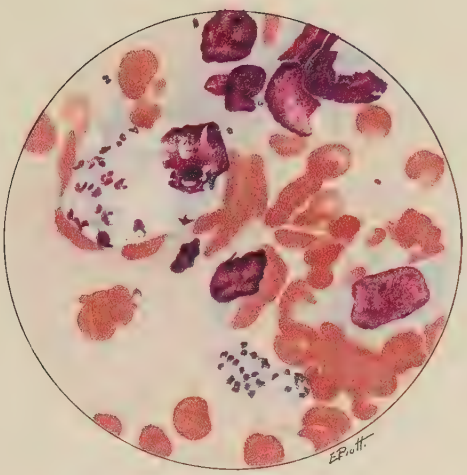


Fig. 1

Fig. 2

PLATE XXXVIII — UTA

Preparation made from a lesion in the early stage of the disease, showing species of Leishmania, the etiological factor.

Fig. 1

Preparation made from a lesion in the early stage of the disease, showing species of Leishmania, the etiological factor.

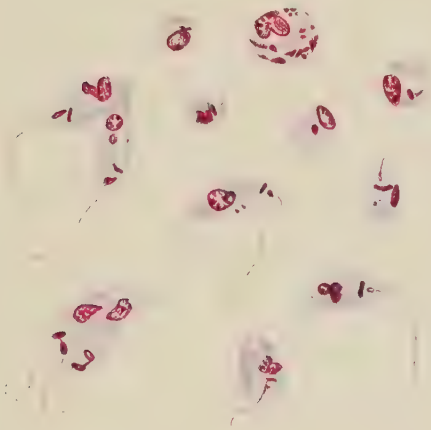


Fig. 2

Flagellate Forms of Leishmania obtained in culture from an Uta case.

PLATE XXXIX — UTA

Fig. 1. — Papular Lesions on Inner Aspect of Dog's Ears. Appearing about one month after inoculation with material from early case of Uta.

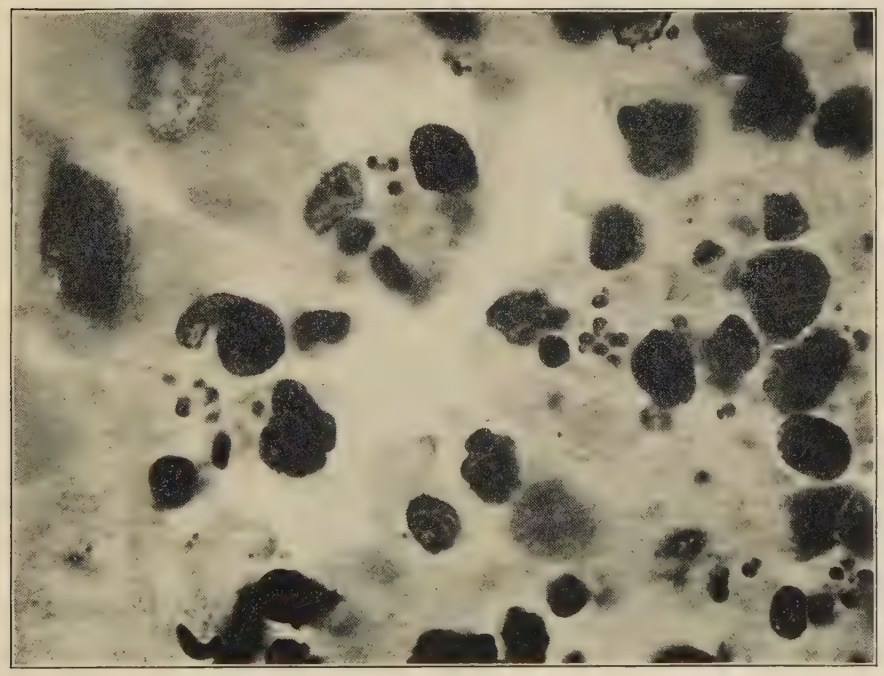


Fig. 2. — Showing Leishmania in a section of the lesion.

Plate XL. — Uta.

visible, the largest measuring 6 × 3 mm., the other three not measuring over 5 × 3 mm. The papules were closely placed. On September 23, Leishmania was observed for the first time in the lesions. The parasites were very difficult to find. The lesions were eventually excised and other animals inoculated, but without result.

VII

GUAYAQUIL

Sanitary Conditions and Prevailing Diseases

The City of Guayaquil (Plate III, page 7) is situated two and one-half degrees south of the equator, on the north bank of the Guayas river, about forty miles from its mouth, and just below its formation by the junction of the Rio Grande and Daule rivers. The land upon which almost the entire city is situated is flat, and has an elevation of only a few feet above the level of the river. At the extreme right, or northern end of the town, there is a low range of hills about five hundred to six hundred feet in height, on the most eastwardly of which is situated the small *barrio* of Las Penas. To the west of the city is situated another range of hills of about the same or slightly greater height, upon which, however, there are no dwellings, but which is covered with a moderately dense forest. The banks of the river on the opposite side of the town are low, and covered with thick tropical verdure. The population is estimated at 80,000, though it is difficult to believe that there is actually so large a number of people living in the town. The city has been destroyed twice by fire, and largely as a result the present streets usually are of broad construction. Only comparatively few of them are paved at all, and these have rough, uneven stones. In many places there are intervals of several feet between the stones, and in many others the stones have become partially sunken, so that following a rain the water collects in the depressions. The traffic over them is necessarily much slower and rougher than over the remaining dirt roads. During the rainy season, however, many of the streets are entirely covered with water; while during the dry one the gutters on each side of them are filled with stagnant water containing decayed vegetable and animal

matter, and frequently covered with algae. Open drains (Plates XLII and XLIII) may be observed in the streets of the greater part of the town, and in them it is common to find mosquito larvae. The general hygiene of the city is bad. In the poorer districts the streets are usually in a very dirty condition. Partially decomposed animals, such as dogs, cats, birds and vultures, sometimes may be seen lying in the streets, as well as piles of other decomposing organic material. In these portions of the town the houses are without closets, and the people frequently defecate openly in the streets. In the rainy season the faecal material is washed away by the water, while in the dry season the conditions obviously become even more unsanitary. The natives apparently, in addition, throw a large amount of offal and rubbish from their houses into the streets.

To the northwest of the town there is situated a large open plain several hundred yards square, and about four feet below the level of the streets which border it. It is surrounded on three sides by dwelling houses, and on the fourth the General Hospital and cemeteries face it. In the rainy season this plain is partially under water, and furnishes an excellent place for the breeding of mosquitoes, and very numerous mosquito larvae may be found there. Beneath several of the streets in the more prosperous portions of the town there are sewers in which large numbers of mosquitoes were also found to be breeding. Still further to the west of the city is a large *estero* which frequently overflows its banks, and then covers an area of land of about one hundred square yards. About it are situated numerous dwelling houses. Anopheles' larvae were also encountered in the shallow water which surrounds the houses in this district. Vultures are the sanitary scavengers of the city, and one may often see groups of from twenty to thirty of these birds devouring offal in the streets.

The majority of the dwellings in the poorer districts are of bamboo and nipa construction, and most of them are hardly more than mere shacks. In the better districts the houses are usually constructed of wood and are from two to three stories

in height; the second stories project over the sidewalks, and furnish a protection for pedestrians from both sun and rain.

The most important hospitals are the Yellow Fever and Bubonic Plague Hospitals (Plate XLI), situated at the extreme north of the town, within fifty yards of one another; the Military Hospital, situated in the *barrio* of Las Penas; the General Hospital; the Hospital for the Aged and Infirm; and the Hospital for the Insane, all of which are situated on the northeastern edge of the town. To the westward of the General Hospital the large Catholic Cemetery is located, and to the north of the Yellow Fever Hospital the burying ground for the poor.

The prevailing diseases in Guayaquil are yellow fever, bubonic plague, dysentery, hookworm infection, malaria and typhoid fever. The Yellow Fever and Bubonic Plague Hospitals have screened wards. Both of them are located near the banks of an *estero*, the Yellow Fever Hospital being situated between this *estero* and a large drain (Plate XLIII, Fig. 1), which constitutes an excellent breeding place for mosquitoes. The Yellow Fever Hospital has accommodation for about one hundred beds, and the Plague Hospital for at least twice this number. Both of these hospitals are under the direction of Dr. Pareja, who very kindly placed at our disposal all of the material in them, and allowed us to study the cases clinically, and to perform necropsies. Fortunately it was very unusual to find a mosquito in the wards of the Yellow Fever Hospital, although flies and gnats were occasionally encountered there. However, *Stegomyia calopus* was not uncommonly observed about the Bubonic Plague Hospital which, as has already been stated, is situated at a distance of about fifty yards from it. In the Bubonic Plague Hospital there was a small laboratory in which a portion of our clinical and laboratory work was performed. On the occasion of our first visit to the Yellow Fever Hospital we observed nine cases of this disease, and during our entire stay fresh cases were admitted almost every day. We were thus enabled to observe all the important clinical features of the disease; and as well were able to study in the post

Fig. 1. — Plague Hospital.

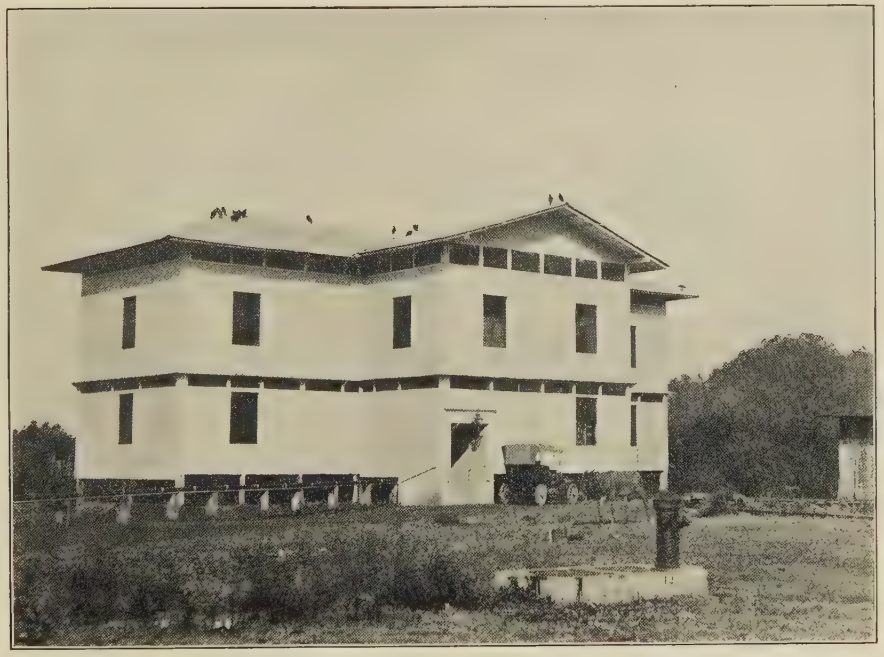


Fig. 2. — Yellow Fever Hospital.

Plate XLI. — Guayaquil.

Fig. 1. — Homes of Yellow Fever Immunes.

Fig. 2. — Open Drain. Breeding Place of Mosquitoes.

Plate XLII. — Guayaquil.

mortem room the important pathological-anatomical changes, and to secure ample material for investigation and teaching purposes.

In the Plague Hospital, on the occasion of our first visit, we observed about a dozen cases of the disease, the majority of which were of a very mild type. The serum treatment was employed in all of the cases, a dose of sixty cubic centimeters being given and repeated in twenty-four hours if the cases did not improve. The mortality from bubonic plague in Guayaquil is not high. Particularly striking is the great mildness of these cases as compared with those observed during epidemics of bubonic plague in the Far East. On the day following our first visit one of the cases died, and we were enabled to perform a necropsy and to verify bacteriologically the diagnosis of the disease. Cultures obtained from the bubo of this case, as well as the bubo itself, were preserved. (Plate XLV.)

In the General Hospital we were particularly struck by the many very advanced cases of uncinariasis which we observed in the wards. Many cases of malaria were also observed there.

Great difficulty is experienced in Guayaquil in the diagnosis of yellow fever. About one-third of those admitted to the Yellow Fever Hospital were found by us to be suffering from malarial infection. The correct differential diagnosis from malaria upon clinical grounds is frequently impossible. Obviously, however, the microscopical examination will give definite information. On the other hand, one must remember that in a city like Guayaquil, where during the year about 95 per cent of the population are said to suffer from malaria, a concomitant infection with this disease and yellow fever may likewise exist, and that even though malarial parasites may be found in the blood, nevertheless the individual may likewise be infected with yellow fever. We observed two such instances while there.

Guayaquil, from a sanitary standpoint, compares unfavorably with some of the notably unhealthy cities of the world.

Milagro. Owing to the reports that cases of yellow fever were continually appearing in Milagro, it was decided to visit this village, and to investigate the sanitary conditions which prevailed there. Milagro is situated on the main railroad line from Guayaquil to Quito, and has an elevation of but forty-two feet above sea level. There are about 6,000 inhabitants in the town, and the number of deaths varies in the rainy season from six to ten every day. The majority of the deaths are said to be due to yellow fever and malaria. Almost the entire population is of the poorer class, many being employed upon the large sugar *hacienda* situated near the town. The village presents a forlorn appearance; most of the houses are of cheap nipa construction. A sluggish but picturesque river about fifty feet in width, flows along the edge of the town. Its shores are covered with bamboo and palm. (Plate XLIV, Fig. 1.) About the dwelling houses in the town there are numerous barrels and cans containing water, and in these receptacles we almost invariably found large numbers of mosquito larvae, and in some instances the eggs of *Stegomyia* were encountered. Many of the dwellings were visited in which sick individuals were found, and we secured a large number of blood specimens. A large number of the people had enlarged spleens; and we also observed many cases of advanced anaemia in addition to the cases of malaria and yellow fever.

Entomological Investigations

On account of its location in a region of great rainfall and high humidity, nearly at sea level and almost on the equator, Guayaquil and its environs support a very extensive and varied insect fauna and in relation to insect-borne diseases there are certain salient features which attract the attention of the entomologist. As has been pointed out, the city fronts along the bank of the Guayas river and extends as a quite thickly settled area for a short distance inland. Beyond, the outlying country spreads out as a large savanna broken here and there by small hills, and by depressions, many of which contain water. Most of this water extends as narrow arms

Fig. 1. — Open Drain behind the Yellow Fever Hospital.

Fig. 2. — Breeding Places of Yellow Fever and Malaria Mosquitoes.

Plate XLIII. — Guayaquil.

Fig. 1. — Milagro, near Guayaquil. A yellow fever district.

Fig. 2. — Surco, Peru, where Uta, Oroya Fever, Malaria, and Verruga Peruviana are prevalent.

Plate XLIV.

or bayous and is ultimately connected with the Guayas river, so that it rises and falls to a greater or less extent in conformation to the tidal ebb and flow in the river. About this water are mangrove swamps some of which line its edges while others spread out over more extensive areas. The region is thus a vast swamp relieved by large grassy areas and scattered hills.

In spite of the vast quantities of stagnant or slowly moving water, mosquitoes at certain seasons of the year are not nearly so abundant as might be expected. In the first place the tidal water which forms an integral part of the Guayas river system is decidedly brackish as evidenced by its molluscan and crustacean fauna as well as by the mangroves. During the rainy season the salinity must fall much lower but evidently cannot well support the larvae of fresh-water insects to any considerable extent. During portions of the dry season Anophelines are practically absent, although a few larvae are found in the confines of the city in broad shallow ditches. Such is far from the condition, however, during the rainy months.

Stegomyia calopus was commonly to be observed in almost any part of the city during the day as well as in the evenings, but in comparison to various places in the southern United States and in the Greater and Lesser Antilles where this species occurs, it could hardly be considered as abundant. The larvae were found in a number of instances breeding in barrels and tubs containing water kept for domestic purposes or for watering animals within the city. Owing to the activities of the municipal health authorities, however, in suppressing such nuisances the breeding opportunities for this species are slightly curtailed. It does not appear that a temporary practical elimination of the yellow fever mosquito from Guayaquil would be an extremely difficult undertaking. Infected *Stegomyiae* are, however, liable to introduction into the city at any time on the numerous small boats or roofed-over rafts which migrate up and down the river between Guayaquil and the smaller towns and settlements further up the river. These also carry

water and could at any time serve as breeding grounds for this mosquito.

A considerable amount of time was spent in visiting various parts of the city to ascertain the opportunities for the breeding of Stegomyia in addition to the aforementioned receptacles in which water is kept. As may be expected from the general contour of the region, there is much standing water in the streets, gutters, open sewers and scattered isolated pools, but none of these places were found actually to harbor Stegomyia larvae, and the majority were obviously unsuitable for this purpose. The following abstracts from notes made at the time bring out this point quite clearly.

"At this time, the 18th of May, the dry season has begun and conditions in the confines of the town are evidently very different from those which prevail during the rainy season. To the south the streets are all very low and there is even now a fair amount of water stagnant in the streets. This is mainly in the form of drains which run out from the houses and also a few pools and gutters that have algae growing in them, showing that they are of long standing. From the cracked condition of the clayey surface-soil it is very plain that in the rainy season there is practically no dry street. At the present time mosquitoes are not very abundant and this is readily accounted for by the rapidity with which the rain disappears. Although it rained heavily two days ago the streets are dry today. In the paved street in front of the hotel there are numerous large depressions containing water due to the large size of the irregular paving blocks; these become dry almost twenty-four hours after the rain has ceased and on account of traffic cannot possibly harbor larvae in the rainy season. The water from the sewers opening into the street appears to be too dirty for Stegomyia so that these must come from receptacles kept in or about the houses."

It appears that the opportunities for Stegomyia to breed are in many places limited, but nevertheless this mosquito was the commonest species to be observed in the city. In con-

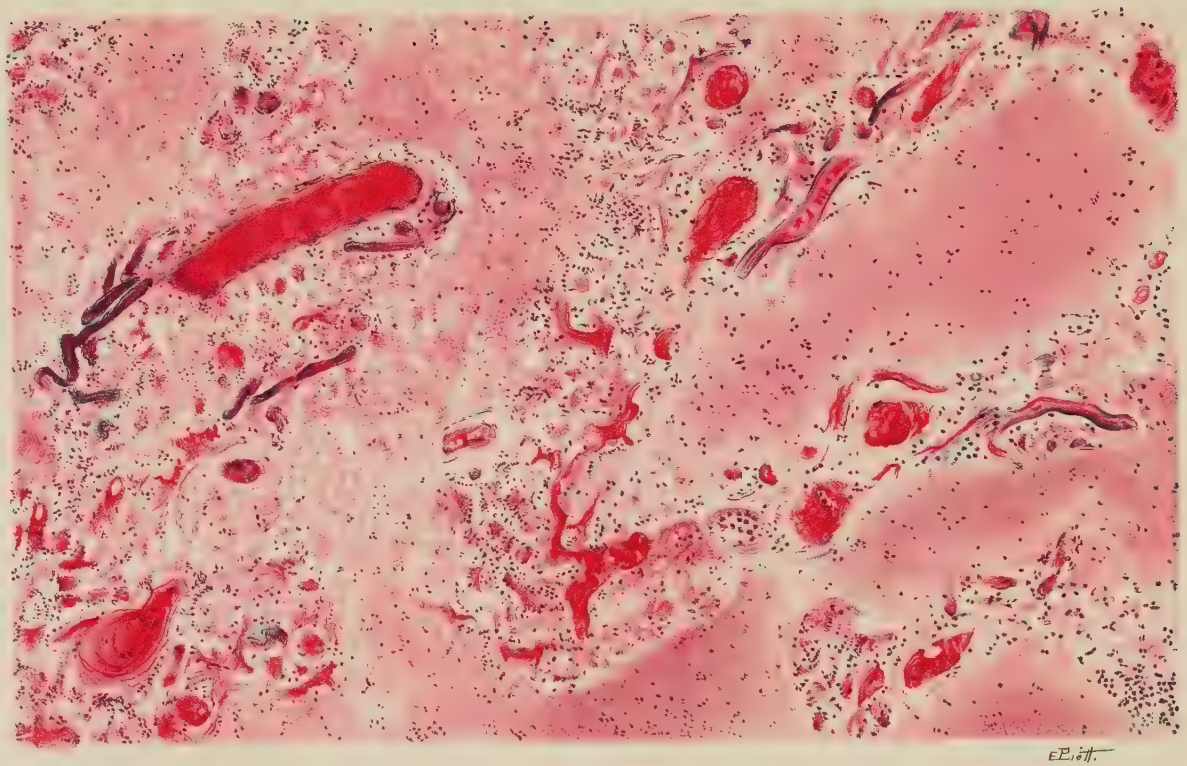


PLATE XLV — SECTION OF BUBO, IN PLAGUE.

formity with its habits as they have been observed elsewhere, the mosquitoes were not numerous during the day, and were observed to bite almost exclusively in poorly lighted rooms, very rarely in a rather bright light. They were very frequently attracted to a dark hat if it was hung on the wall of a room. They bite mainly about the ankles when there is light. At night also they bite most frequently about the ankles, but at that time about the neck and other exposed parts of the body as well.

This brings out the fact, already observed elsewhere, that a large Stegomyia population is not necessary for the maintenance of a constant incidence of yellow fever in a community. The typical domestic habit of the species coupled with the undoubted great length of its adult life render the spread of infection easy through the agency of a small mosquito population. The authorities do not remove cases of yellow fever to the isolation hospital promptly at the onset of the disease, and such cases though later screened are free to infect many mosquitoes just at the period when such infection is possible. Even in this community composed mainly of immunes, the seasonal prevalence of Stegomyia bears a marked relation to the incidence of yellow fever and it is evident that any reduction of mosquitoes must have a prompt effect in lessening the prevalence of the fever.

Unfortunately we were not able to make any observations on fleas in their relation to bubonic plague. According to the municipal hospital records, this disease is even more common than yellow fever, with about the same mortality. It is said to be spreading rapidly inland, particularly along the railroad, and there should be excellent opportunities for work concerning the distribution of rat fleas, their seasonal prevalence and relation to the dry season. From data gathered by the Servicio de Sanidad Publica,[1] the minimum incidence appears to fall two or three months earlier than that of yellow fever, that is to say from April to June. This fact is very marked and is extremely interesting in connection with the

[1] Informe Direcc. Serv. Sanidad Publ. Guayaquil, 1910 and 1912.

recent work of Bacot and Martin on the seasonal prevalence of Plague in India,[1] where they believe that the sudden onset of the dry season causes a great decimation of fleas through certain peculiarities of their feeding habits.

Outside the city in the savannas and on the slopes of the hills where there were no or very few habitations Stegomyia as might be expected was practically absent, but mosquitoes were abundant and annoying. These which were almost exclusively *Mansonia titillans* (Walker) occurred sparingly about the edges of the inhabited area, but were typically restricted to the uninhabited areas.

Several other species were taken in lesser numbers, as follows:

Aëdes scapularis Wied.
Aëdes oswaldi Lutz.
Aëdes ? taeniorhynchus Wied.

As mentioned above, the absence of Anophelines was very noticeable at that time of the year and contrasts sharply with their great abundance during the rainy season which was reported to us by several competent observers who had lived in Guayaquil for a number of years.

A number of insects of other groups were secured during our stay in Guayaquil and some of the more interesting of these are reported upon in the appendix to this report on page 209.

[1] Bacot and Martin: J. Hygiene, Plague Suppl. III, 1914, pp. 423–429.

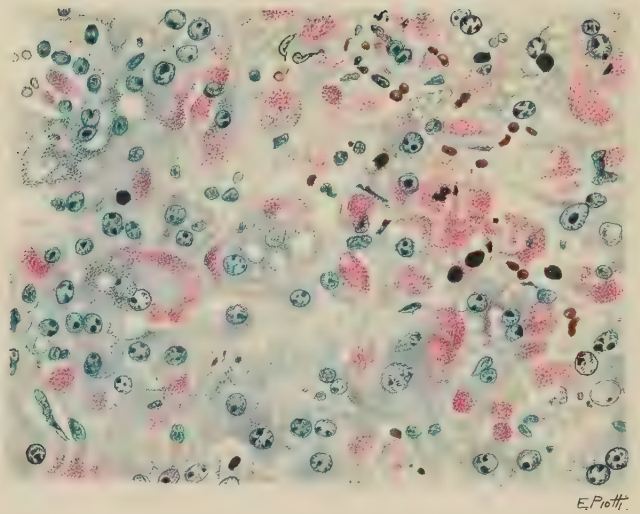


PLATE XLVI — LIVER IN YELLOW FEVER

VIII

YELLOW FEVER

In 1909 Seidelin [1] described bodies in the blood of yellow fever patients which he now regards as parasites, and the cause of the disease. Regarding these he writes, "During the earlier days in which the initial fever occurs I have found only extremely small forms with a minute chromatin point, and a generally feebly stained blue protoplasm. The chromatin is not very red, but rather dark violet. A little later during the reduction of the temperature, when this occurs on the third or fourth day, or in the period of reactive fever, there are still seen the same small forms; these are usually few in number, but there are in addition others, a little larger, which, whilst they consist chiefly of a rather deeply stained blue protoplasm, contain a still very small, but more purple-stained chromatin point. Still later, in the last days of the fever, and, as it seems, far into the convalescent stage, we found in the corpuscles chromatin granules, these being well stained single or double, round or pear-shaped, and with a very small border of protoplasm or quite free of protoplasm. All these forms are intraglobular."

In subsequent papers [2] he reported upon finding this parasite in over 100 cases of yellow fever. As these observations had not been confirmed at the time that we were in South America upon this expedition, we determined to study the blood in this disease particularly with reference to the presence of these bodies, and also to attempt to cultivate them if possible. A summary of the histories and necropsies of some of the cases in which blood examinations were made, and from which cultures were taken with reference to this question follow.

[1] Seidelin: Berl. klin. Wchnschr., 1909, No. 18, p. 821.
J. Path. & Bacteriol., Edinb. & Lond., 1910, xv, 282.
[2] Yellow Fever Bureau Bull., Liverpool, 1912, ii, 123.
Ibid., 1913, ii, 255.

Yellow Fever Case 8. May 22, 1913.

Patient an adult, seen first on May 21, on the sixth day of the disease. Temperature on admission 101°. 1 F. There is general jaundice, particularly of the conjunctiva. The pulse is slow. The urine contains much bile pigment; and on shaking it in the test tube a foam persists on the surface due to the albumin which it contains. The urine is scanty, less than 600 c.c. being passed in twenty-four hours; it contains about one and one-half grams of albumin. During the following twenty-four hours the patient developed marked epigastralgia and hematemesis and anuria developed. He became delirious, the temperature fell to below normal and he died on the morning of May 22. Microscopical preparations of the blood and blood cultures were made May 21.

Autopsy. Five hours post mortem. Conjunctivae greenish yellow in appearance. Skin presents a general yellowish icteric tinge. Slight post mortem lividity over dependent parts. Rigor mortis well marked. Pupils equal 8 mm. in diameter. Subcutaneous fat is of an icteric tinge. On opening the abdominal cavity, small black punctate hemorrhages from 1 to 4 mm. in diameter, may be seen situated in the serosa of the small intestine. They are sharply circumscribed. The viscera are all bile-stained. There are about 100 c.c. of greenish-tinged clear fluid in the abdominal cavity.

Heart. The pericardial cavity contains a small amount of clear greenish-yellow fluid. There are no ecchymoses beneath the epicardium or endocardium. The heart is of normal size. Valves all normal. Chambers contain fluid and partially clotted blood, together with some greenish-tinged chicken fat thrombi. Heart muscle pale in color.

Lungs. The lungs are both voluminous. *Left.* Base of the lung is bound to the surface of the diaphragm by old adhesions. These are easily separated. The anterior surface in general is pinkish gray in color, and the lung contains air throughout. At the apex there are a number of ecchymoses, some discrete, 1 to 3 mm.; others confluent, forming patches of about 1 cm., or larger, in diameter, of a dark blue or almost purple color. Over the lower lobe there are also numerous punctate hemorrhages beneath the pleura. The posterior and lateral surfaces show diffuse dark bluish or purple areas, covering the greater part of this portion of the lung. The bronchial lymphatic glands at the base of the trachea are dark purple from the hemorrhages which have occurred in them. They are not markedly swollen. The bronchi contain frothy mucus. The mucous membrane is deeply reddened, the vessels injected. The cut surface of the upper lobe is pinkish red in color, and contains air throughout, except in a small calcified area, 0.5 cm. in diameter, and situated 4 to 5 cm. from the apex. The lower lobe is much congested and very oedematous. The color is red. Dark red or purplish areas, measuring from 2 to 3 mm. in diameter are scattered throughout. Their margins are irregular, not round. They are not wedge-shaped, and the surface is not granular. *Right.* The right lung presents in general the same appearance as the left. In general it is pinkish gray in color, and there are a few punctate and diffuse hemorrhages beneath the pleura. The lung is voluminous, the surface smooth. The base is in general dark blue or purple in color. The color of the lower lobe is more variegated. The cut surfaces of the upper and middle lobes are in general normal in appearance. The lower lobe shows considerable congestion and oedema, and has a mottled appearance, due to the hemorrhages which have occurred. The bronchi show an appearance similar to that encountered in the left lung. The cut surface of the lung is smooth, and there are a few early hemorrhagic infarcts.

The Spleen. Measures 15.5 × 6.5 × 3 cm. It is of dull brownish purple color, and of moderately firm consistence. The surface is in general smooth, but there

are a few areas of chronic perisplenitis. Cut surface is dull brown red with a purplish cast. The follicles are moderately distinct and some slightly swollen. The trabeculae are not prominent.

The Liver. Measures 32 × 15.5 × 7 cm. It is of moderately firm consistence, but tears easily. The general color is dull yellowish brown. The surface is smooth. The vessels near the tip of the left lobe are prominent. In the superior and anterior surfaces of the right lobe there is an area measuring 1.5 cm. in diameter, with irregular margin, and presenting a yellowish peripheral zone, with a dull dark brown central area. The central portion is not uniform in color throughout, but shows a number of yellowish points 1 mm. in diameter. In general the markings of the lobules are distinct. On cut section the liver presents a mottled yellow and red appearance; the red lobules are sometimes surrounded by dull brown areas. Other sections show a coarser red mottling. Still other areas show a fine pale yellowish mottling.

Organs of the Neck. The tongue shows numerous superficial erosions at the edges. The edges of the tongue are clean, but in the center there is a dark red coat. The oesophagus appears normal throughout. There is a large *Ascaris lumbricoides* lying partially in the oesophagus and partially in the pharynx. There are minute ecchymoses in the trachea. The tonsils are filled with a dry cheesy material. The bronchial lymphatic glands are injected, and of a dark red or purple color.

The Kidneys. Measure 12.5 × 6.5 × 3.5 cm. There are a few diffuse hemorrhages in the capsule near the pelvis. The capsule is slightly adherent, and tears on removal. The color of the surface is yellowish brown. Cortex measures 1.4 mm. The glomeruli are not prominent. The *Adrenals* appear normal.

The Stomach. Measures 25 × 15 cm. It is filled with reddish black bloody material. The mucous membrane is swollen and more or less injected. The duodenum also contains partially fluid blackish material. The mucous membrane of the small and large intestine is in general normal in appearance. There are several *Ascaris lumbricoides* in the small intestine, and numerous *Trichocephalus trichiurus* in the cecum. In the walls of the small intestine, beneath the serosa, there are numerous punctate (from 1 to 4 mm. in diameter), black hemorrhages. Portions of the stomach are preserved for museum specimens.

The Bladder. The mucous membrane of the bladder appears normal. There is a small amount of cloudy urine.

The Brain. The meninges are slightly injected. The ventricles contain a small amount of clear fluid. The other organs appear normal.

Bacteriology. Cultures were made from the blood during life and from the pericardial fluid, heart, spleen and liver, at autopsy, and microscopical preparations from the heart, spleen, liver and kidneys.

Histological Examination

Heart. No pathological process noted.

Lung. There are extensive areas of hemorrhage throughout the greater portion of which the blood is laked, and large bacilli are noted scattered throughout these portions. There are here also small foci of infiltration with polymorphonuclear leukocytes. Many of these contain bacteria.

Small Intestine. Poorly preserved, the mucosa from the villi being necrotic. The section shows no prominent abnormal feature.

Stomach. Few minute foci infiltrated with polymorphonuclear leukocytes. In certain areas a more diffuse infiltration of lymphoid and plasma-cells.

Oesophagus. Normal.

Tonsil. Epithelium of the crypts is infiltrated with leukocytes. The crypts contain large masses of bacteria and detritus. In places there are defects in the epithelium associated with local necrosis. There are occasional small hemorrhagic areas, and the follicles are to a large extent replaced by large, pale staining cells many of which are phagocytic.

Tongue. Section shows several small superficial clumps of organisms which appear as thick rods arranged in chains.

Liver. The greater portion of the liver is necrotic and the liver parenchyma is replaced to a large extent by blood. The living cells which constitute only a very insignificant part of the organ are grouped for the most part around the larger vessels and are usually present in larger amount around the central vessel than around the portal canal. Mitotic figures are frequently found within liver cells. Nearly all cells are markedly vacuolated and as the degenerated areas are approached the cells are found distended with vacuoles. Intermingled with these are red staining necrotic cells and granular material representing the remains of those which have undergone further disintegration. The blood is in part distributed in spaces formerly occupied by the liver columns. Although mitotic figures are numerous, there is no evidence of an arrest of the destructive process or of regeneration.

Pancreas. There is no evidence of destructive process, but zymogen granules are absent throughout the entire section.

Spleen. Lymph-follicles are inconspicuous. In several there are branching masses of dense fibrinoid material situated within their substance. No pigment.

Lymph-Node. Contains a large amount of carbon pigment. No lesion noted.

Kidney. Many of the smaller tubules show casts of hyaline material staining either pinkish or bluish with eosin methylene blue. The glomeruli are considerably congested as is also the greater portion of the medulla. The convoluted tubules are somewhat distended; the epithelium shows no marked evidence of degeneration.

The *Cerebrum* and *Cerebellum* show no lesions.

Anatomical Diagnosis. Inflammatory foci situated in hemorrhagic areas of lungs. Slight acute gastritis. Slight tonsillitis. Ulceration of tongue. Degeneration and necrosis of liver parenchyma. Loss of zymogen in pancreas. Acute splenitis with fibrinoid substance in follicles. Moderate acute nephritis.

Yellow Fever Case 20. Woman, aged thirty-six years. Temperature on admission 103°.2 F., pulse ninety-four. There is practically complete anuria. Marked epigastralgia. The tongue is clean, the gums reddened and swollen. There is marked icterus. The spleen is not enlarged. The liver is painful. There is marked metrorrhagia. She was given sodium bicarbonate intravenously. The temperature fell to below normal and she died the following day, May 23.

Autopsy. There is marked post mortem rigidity. About 3 cm. above and behind the right knee joint there is a purplish area 2 cm. in diameter. The general color of the skin is yellow; the conjunctiva greenish yellow. The muscles are normal in appearance, and of a dull red color. The subcutaneous tissues are bile stained. On opening the abdominal cavity numerous hemorrhages in the peritoneum measuring from 2 to 3 mm. to 1 cm. in diameter are visible. The vessels in the serosa of the transverse colon are injected, and there are a few pinpoint sized hemorrhages. There is also an extensive hemorrhage at the base of the mesentery. The peritoneal cavity contains about 200 c.c. of clear greenish yellow fluid. The liver projects 4 cm. below the costal margin. There are some hemorrhages over the greater curvature of the stomach.

Heart is of about normal size; contains fluid blood. No hemorrhages in the epi- or endocardium. There is a soldiers spot 1 cm. in diameter over the anterior

surface of the heart. The endocardium has a slight yellow tinge. The heart muscle is paler than normal.

Lungs. The lungs are voluminous, and contain air throughout. There are old adhesions over the posterior surface of the left lung. There is no fluid in the pleural cavity. In the superior lobe of the left lung there are numerous hemorrhages measuring from 1 to 3 cm. in diameter. The bronchi are dull red in color throughout, but the bronchioles are normal. The cut surface is pale pink. There is no oedema. In the left lung there are several small hemorrhagic infarcts near the apex, 6 to 8 mm. in diameter. Tracheal mucosa is not greatly reddened. The bronchial glands are dark red or black in color.

Spleen. The spleen measures 10 × 5.5 × 2.5 cm. It is small and tears easily; dark red in color. Pulp moderate in amount. Trabeculae prominent. Follicles not visible.

Liver. The liver is opaque gray with yellowish green cast, measures 22 × 15 × 7 cm. There are a few small punctate hemorrhages, irregular in outline, scattered over the surface from 1 to 3 mm. in diameter. The liver tissue is friable. The lobular margins are indistinct. Cut surface shows a fine mottled brownish red bronze. Gall bladder is distended; diffuse hemorrhages in its wall. The duct is patent.

Kidneys. The fat about the kidneys is greenish yellow in color. There are a few hemorrhages in the capsule 3 mm. in diameter. The left measures 11 × 6 × 4 cm. *Right* 9.5 × 6 × 4.5 cm. Pyramids dark in color. Cortex 7 to 8 mm. Glomeruli distinctly visible. Capsule tears in stripping; is slightly adherent. Color of kidneys bluish red.

Aorta shows no atheroma. The *bladder* is contracted and empty. The mucous membrane pale.

The Uterus. Measures 8 × 5 × 3.5 cm. and contains a small amount of clotted and fluid blood.

The *pancreas* appears normal.

The mucous membrane of the *stomach* is swollen; reddish gray in color; and there are several irregular hemorrhagic areas not over 1 cm. in diameter, with irregular margins. There are also hemorrhages in the walls of the ascending *colon.* The *small intestine* in the upper part is full of reddish black fluid material. In the *cecum* and *large intestine* the mucous membrane is grayish. The follicles are not swollen. Near the stomach the mucous membrane shows some hemorrhages.

Bacteriology. Cultures were made from the blood during life and from the heart, spleen and liver. Microscopical preparations were also made from the blood during life and from the visceral organs at autopsy.

Histological Examination

Heart. No marked lesion apart from presence of vacuoles in muscle fibers. *Aorta.* Negative. *Lung.* Negative. *Trachea.* Normal except for hemorrhage in a small lymph-node lying in its outer wall. *Stomach.* Numerous foci infiltrated with lymphoid and plasma-cells associated with which are large numbers of coarse rather thick bacilli with rounded ends. These, however, are not confined to the foci of inflammation: the superficial portion of the mucosa is markedly congested. There are occasionally large masses of bacteria situated in the superior portion.

Small Intestine. The villi are denuded of epithelium. There is no demonstrable lesion.

Liver. Large proportion of the liver parenchyma is degenerated or necrotic. The necrotic cells appear red and granular. Portions of the liver cells also show

this red granular material. Most of the liver cells are greatly enlarged and vacuolated. There is no pigment except a few granules which are seen within liver cells. The better preserved portions of the parenchyma show a more or less irregular distribution, occurring both around the portal canals and central vessels. There is no marked process of regeneration. Small foci of lymphoid and plasma-cell infiltration occur for the most part in the region of the portal canal.

Pancreas. Certain of the gland alveoli show small groups of degenerated cells. Practically no zymogen granules.

Adrenal. There is a marked degree of parenchymatous degeneration affecting chiefly the medulla. There is considerable congestion present.

Kidney. Convoluted tubules somewhat dilated with granular excretion. Many deeply stained hyaline and granular casts. The glomeruli are moderately injected, and there is rather marked injection of the medulla with several hemorrhagic areas.

Spleen. Follicles not atrophied and show no fibrinoid material. The pulp contains considerable blood. No pigment present. Large numbers of phagocytic cells appear in certain areas. These contain red corpuscles and red staining granular material; they show only an occasional granule of pale pigment.

Lymph-Node. Peripheral sinuses filled to large extent with polymorphonuclear leukocytes and a small number of phagocytic cells. Lymph-follicles show small areas of degeneration centrally situated.

Anatomical Diagnosis. Acute parenchymatous degeneration of the liver. Congestion of gastric mucosa. Considerable phagocytosis in spleen.

Yellow Fever Case 21. Patient twenty years of age, from Riobamba. Seen on the fourth day of the disease. Temperature on entrance 103°. 2 F. pulse 58. Patient presented marked jaundice of the skin and conjunctivae. The urine varied between 900 and 300 c.c. The albumin in the urine varied between 1 and 2.5 grams. There was pain over the abdomen. The patient developed hematemesis and coma, the pulse rose to one hundred, and he died on the 27th of May.

Cultures were made from the blood on May 25th.

Autopsy three and one-half hours post mortem. Rigor mortis moderate. Livor mortis also moderate. Entire skin jaundiced. Slight lividity about neck and forehead. Pupils equal. On opening the abdominal cavity the peritoneum is smooth. The mesocolic lymph-nodes are markedly reddened: cavity contains 70 c.c. clear yellowish fluid. Bladder distended. There are two ecchymoses 2 cm. in diameter on the anterior surface. The pericardial fluid not increased. There are greenish yellow chicken fat thrombi in the heart. Valves normal. Muscle pale brown in color. Extensive hemorrhages in the left ventricle beneath the endocardium. These are superficial, not invading the muscle, and measure from 1 to 2 cm. in size.

Lungs. Voluminous. Pinkish gray anteriorly. Left lung, the surface is smooth. There are numerous hemorrhages scattered over the posterior and lateral surfaces. These have regular margins, and measure 2 mm. to 1.5 cm. They are dark purple or black in color, and extend into the depth for a distance of about 7 mm. *The bronchi* are much reddened. The cut surface of the lung is dark red, with a few hemorrhagic areas. The right lung presents a similar appearance to the left. The bronchial lymph-nodes are dark red.

The Spleen is not enlarged, and measures $11 \times 7 \times 3.5$ cm. The surface is smooth, dark blue or purple in color. On cut section the follicles are not visible. The trabeculae may be distinguished. The cut surface is dark red in color.

The Liver. The surface is smooth; mottled yellowish red and brown. Cut surface lobules not distinct. There is a red and greenish yellow mottling. In

places the lobular markings have an opaque peripheral zone of a yellowish gray color, within which is a reddish ring surrounding a gray center (pin-point in size). The gall bladder is practically collapsed, and contains a small amount of viscid mucoid greenish bile.

The Kidneys. Capsules strip rather easily, but the surfaces tear slightly. The surface vessels injected. Cortex measures 7 to 11 mm. Dull brownish red with yellowish cast. Glomeruli not prominent. Size 11.5 × 7 × 4.4 cm. Cut section reddish yellow. In the right kidney are several small brownish red infarcts measuring from 2 to 3 mm. in diameter at the base. They are wedge-shaped.

The Stomach is filled with reddish black blood. There are round worms in the stomach and oesophagus. There are very numerous hemorrhages in the mucous membrane 4 to 5 mm. in diameter.

The Aorta is smooth.

The Bladder is distended with urine, which is cloudy and amber colored. The genital organs appear normal, as does the thyroid. *Large intestine* largely filled with bluish black faeces. No ulcerations. No hemorrhages. *Trichocephalus* in the cecum. There is dark green material in the small intestine, and no ulcerations. The solitary follicles are not prominent.

Bacteriology. Cultures were made from the heart, spleen and liver.

Histological Examination

Heart. Certain fibers appear somewhat swollen. Nuclei large and vesicular. *Lung.* Areas of hemorrhage. *Stomach.* No lesion except superficial portion of mucosa somewhat injected in places.

Liver. Changes are practically identical with those seen in Yellow Fever Case 20. Only a very small amount of parenchyma takes a bluish stain. No pigment is present. *Pancreas* shows no lesions; the glands stain blue throughout; and show no zymogen granules. *Kidney.* Glomeruli are considerably injected, the capsules somewhat distended. Only an occasional cast is found in the collecting tubules. *Testes.* Present no lesions. Spermatogenesis apparently arrested. *Prostate.* No lesion. *Spleen.* Follicles atrophied. Spleen contains a moderate amount of blood. Numerous large phagocytic cells containing red blood-corpuscles. No pigment.

Lymph-node. There is some degeneration and phagocytosis centrally situated in the follicles.

Anatomical Diagnosis. Acute parenchymatous degeneration of the liver. Slight congestion in the gastric mucosa. Acute nephritis.

Yellow Fever Case 26. Patient in coma May 28. There is dark black blood about the lips. There is an odor of decomposing straw from the breath. Pulse is slow, seventy to the minute. Temperature is 100°. 4 F. Spleen is not enlarged. The conjunctivae are very yellow, and the face tinged with yellow. Pupils do not react alike. There is blood on the hands from about the mouth. The urine contains albumin. Hematemesis increased and he gradually sank, and died May 29.

Microscopical preparations of blood and cultures taken during life.

Autopsy two hours after death. There is well marked rigidity of the lower limbs, and moderate rigidity of the upper. Face has a distinct icteric tinge. There is slight icterus of the trunk. Dried blood on the hands, forearms, thighs, ears, nose and lips. Livor mortis over dependent parts. Subcutaneous fat moderate in amount, bile-tinged. Muscles of a good red color. On opening the chest the lungs are voluminous, the heart distended with fluid blood. There are a few

discrete hemorrhages in the mesentery, about 1.5 to 3 mm. in diameter. The mesenteric glands are slightly reddened and swollen. Mesocolic glands more markedly reddened. No petechiae over the heart. Pericardial fluid normal and clear. The chambers of the heart contain fluid blood, and are entirely free from clots. No petechiae of endocardium; valves normal except all bile-stained. Muscle pale and soft.

Lungs. The lungs are voluminous; anterior surfaces pinkish gray in color. There are old fibrinous adhesions on right side anteriorly, laterally and posteriorly. The *left lung* at the apex of the lower lobe shows a large hemorrhagic area 9 × 5 cm. in diameter; margins irregular, and outside of this about one dozen small hemorrhages 1 × 5 mm. in diameter. The hemorrhages frequently follow the lobular markings. At the apex of the lung there are hemorrhagic wedge-shaped areas. The remainder of this lobe, as well as the upper lobe, is entirely free from hemorrhages. In the center of the large hemorrhagic area on palpation firm nodules may be felt in the substance of the lung beneath the pleura. The *right lung*, anterior surface is entirely free from hemorrhages. Lateral and posterior surface shows a large diffuse hemorrhage beneath the pleura. In the upper lobe the hemorrhagic areas measure when discrete from 3 to 8 mm., and when confluent several cm. in diameter. The middle and lower lobes show also numerous discrete and confluent hemorrhagic areas. On opening the bronchi the larger ones show a moderate injection of the vessels, and contain reddish frothy fluid. In the *right lung* apart from the chronic fibrinous pleurisy previously described, and the hemorrhages in the lung substance, the lung tissue is normal. On cut section the lower lobe, and portion of the middle lobe, there is a large hemorrhagic area which is found to extend in depth to the hiatus of the lung, and indeed to occupy the greater portion of the entire lower lobe. The lung tissue in the hemorrhagic area is firm to the touch, and contains but little air. It is moderately oedematous. On further section of the lung there are numerous discrete and confluent hemorrhagic areas, measuring 2 to 3 mm., which are scattered throughout the lung tissue. These areas have irregular margins. On section of the *left lung* through the hemorrhagic areas the hemorrhages are found to extend into the lung tissue for a depth of 2 to 3 cm. The more nodular areas which may be felt beneath the pleura, as one passes the hand over the surface, are found on section to consist of distinct hemorrhagic infarcts, and over these nodules of infarction the pleura is arched. (Sections through the hemorrhagic infarcts saved in Zenker's solution.) Otherwise the lung appears normal; tissue everywhere containing air, and is not congested or oedematous. The bronchial lymphatic glands at the base of the trachea are black from the resulting hemorrhages which have occurred in the gland.

Spleen. The spleen is of about normal size, measures 13 × 8 × 3 cm. Surface smooth, dark purple in color. On cut section the pulp is rather soft, and the follicles indistinctly visible. The trabeculae are visible in places.

Kidneys. The capsules strip with slight difficulty, tearing on separation. The stellate veins are injected, and form a marked contrast in color to the distinctly yellow fatty cortex. There is practically no red color in the cortex. On cut section the cortex measures from 7 or 8 mm. to 1 cm. in thickness. The surface of the cut section is walnut colored. The vessels of the pyramids are moderately injected. The glomeruli are not visible. The vessels of the pelvis are injected. *Adrenals* are normal.

Liver. Surface is smooth, consistence is soft, and tears rather easily. Surface presents a mottled appearance. In general it is brownish red and yellowish. The reddish areas are apparently due to the dilated vessels. There are a few yellowish areas beneath the surface of the capsule, measuring 1 to 2 mm. in diameter. On

cut section these areas are sharply circumscribed, pure yellow in color, and extend for a depth of 1 mm. into the liver substance. Marked by scissors for identification. On cut section there are several other of these yellowish areas. One found in the depth of the liver protruding slightly from the cut surface.

On cut section of the liver the surface is distinctly uneven, and presents a slightly nutmeg appearance. The uneven appearance is caused by small circumscribed raised areas, sometimes confluent, from pin-point to pin-head in size, which are of a brown color, and which are situated upon a grayish background. These small islands of tissue are surrounded by a narrow red zone. In the depth of the liver another of these yellowish areas is found, measuring 10 × 6 mm.

Stomach is filled with reddish black material. The omentum contains numerous small hemorrhages. The mucous membrane of the stomach is covered with blackish mucoid material, on removal of which the mucosa appears grayish yellow with small dark red lines and points due to injection of the vessels. Mucous membrane of the stomach is slightly swollen, but otherwise appears normal. The surface of the *intestine* is bile-stained as already noted. The mesocolic glands are more markedly reddened than the mesenteric glands situated at a distance. On opening the lower portion of the large intestine it is found to be practically normal and to contain normal faeces. Several feet above the rectum there are several small hemorrhages about the peritoneal vessels. Further up the large intestine is filled with a reddish brown material which has a very foul odor. This condition extends to the large intestine as far up as the cecum. On scraping off this reddish brown material the mucous membrane appears practically normal. In the neighborhood of the cecum, however, the mucous membrane is profusely reddened throughout. This process does not extend beyond the valve, the mucous membrane of the ileum appearing normal as well as its contents. The solitary follicles are distinctly visible, but not swollen. The Peyer's patches are also not swollen. In the jejunum there are evidences of altered blood. The *pancreas, bladder* and *genital organs* appear normal. The other organs also appear normal. There are some erosions at the edges of the tongue, and slight injection of the mucous vessels of the trachea.

Bacteriology. Cultures were made from the heart, spleen, liver and kidneys and also microscopical preparations.

Histological Examination

Heart. The muscle fibers show one or more vacuoles situated in the central portion suggestive of fat.

Lung. Areas of hemorrhage. In one such area there is a mass of dense cellular exudate filling the bronchus, and the alveoli are filled with polymorphonuclear leukocytes and large endothelial cells as well as red blood-corpuscles.

In another section some of the similar vessels show thrombi; the appearances being those seen in infarctions.

Stomach. Presents no lesion.

Large Intestine. Appears normal.

Liver. The larger portion of the liver parenchyma is destroyed. The remaining liver cells are grouped chiefly around the portal canals and to a lesser extent around the central veins. Towards the periphery of the normal areas the cells are more markedly vacuolated than elsewhere, and many of the degenerating cells stain red and appear somewhat granular. Distributed throughout the degenerated and necrotic portions there is a considerable amount of pigment situated to a large extent within the phagocytic cells and suggesting complication with malaria. Many of

the vacuolated liver cells are distended to huge proportions, and mitotic figures are present. There is a moderate amount of pigment situated within the bile capillaries. The blood is distributed throughout the necrotic areas, and it is not always confined in well defined blood-vessels. There are syncytial like masses in the region of the portal canals, possibly an attempt at regeneration on the part of the bile ducts, although they have much the appearance of giant-cells in certain places. There are also numerous foci infiltrated with lymphoid and plasma-cells, somewhat irregular in distribution, but more frequently near the portal canal.

Pancreas. There is no inflammatory or destructive change observed. The glands throughout are devoid of zymogen granules. Rounded globules occur within the glandular epithelium in certain areas.

Adrenal. Parenchyma intact and cells non-vacuolated. There appears to be some oedema of the interstitial tissue.

Kidney. Glomeruli slightly injected. Epithelium of the convoluted tubules shows granular and somewhat vacuolated cytoplasm. There is an occasional hyaline cast. In the medulla there are several minute foci of hemorrhage.

Bladder. Presents no lesion.

Spleen. There is considerable golden-brown and black pigment situated within phagocytic cells characteristic of malaria. The lymph-follicles show regularly a lesion characterized by an accumulation of fibrinoid material in the form of a coarse reticulum and including in its meshes various types of cells.

Lymph-node. Shows large amount of carbon pigment. No marked pathological process.

Anatomical Diagnosis. Infarction of lung. Chronic pleuritis. Extensive degeneration and necrosis of liver. Pigmentation, probably malarial. Acute splenitis with fibrinoid changes in follicles. Cloudy swelling of kidney.

It is not deemed necessary to give the notes of the other cases of yellow fever from which we made examinations of the blood. From the histories given above, it is evident that the diagnosis of yellow fever was unquestionable. In eleven of the most severe cases of yellow fever observed by us in which the cases referred to above are included, numerous inoculations of the blood were made into the ordinary laboratory media such as agar, blood-serum, and egg media, and with the various methods described by Noguchi from the Rockefeller Institute for the cultivation of spirochaetae. Both aërobic and anaërobic preparations of ascitic fluid alone, and of ascitic fluid with agar, or in other instances with fragments of the rabbit's testis, were employed. In yellow fever cases Nos. 8, 20, 21, and 26, inoculations were also made in these media from the heart's blood and the solid visceral organs at necropsy. In none of the cultures was any organism found which appeared to bear any etiological relationship to yellow fever. In Case 21, streptococci were isolated from the lungs and heart's blood.

In the study of numerous fresh and stained blood preparations from the yellow fever cases which were observed, we did not discover bodies which we regarded as of a parasitic nature, and at the same time the cause of the disease yellow fever. In Cases Nos. 22 and 23, young forms of the tropical malarial parasite were found in the red blood-corpuscles. Giemsa's and Wright's stains were both employed, after fixation usually with absolute methyl alcohol and in some instances with ethyl alcohol.

During the past year Macfie and Johnston [1] have reported finding bodies in the red blood-corpuscles similar in appearance to those described by Seidelin. They found these elements in practically every case of yellow fever examined, and also in the blood of guinea pigs, dogs, and rats that had been inoculated with blood from human cases. In guinea pigs these bodies were particularly common.

Fajardo [2] has also very recently reported the presence of these bodies in the blood of one case of yellow fever. Considerable controversy has taken place regarding the nature of these bodies and it appears to be the consensus of opinion today that they are not of a parasitic nature. Agramonte [3] has pointed out that they have been found in many conditions other than in yellow fever, and that the bodies described by Seidelin would not be able to pass through a Berkefeld filter, while the virus of yellow fever certainly does. He also draws attention to the fact that Seidelin has found these bodies in many cases which have presented no signs of yellow fever.

Guiteras [4] has recently objected to the diagnosis of a case of yellow fever in Jamaica in which case the diagnosis was made by Seidelin largely apparently by the finding of these supposedly parasitic bodies. Guiteras points out that the disease is not supposed to exist in Jamaica, and that various clinical features make it very improbable that the case was

[1] Macfie and Johnston: Proc. Roy. Soc. Med., 1914, Jan., vii, No. 3, Medical Section, p. 53.

Yellow Fever Bureau Bull., Liverpool, 1914, iii, 121.

[2] Fajardo: Revista Med. de Yucatan, 1914, Jan., ix, No. 3, p. 53.

[3] Agramonte: Proc. xviith Inter. Cong. Med., Lond., 1913, Sect. 21, Part II, p. 77.

[4] Guiteras: Yellow Fever Bureau Bull., Liverpool, 1914, iii, 110.

one of this disease. Particularly striking in this respect was the absence of fever, the pulse rate, and lack of intense jaundice. Agramonte very justly points out that in connection with the possible inoculations of these bodies into guinea pigs he has seen similar bodies in guinea pigs treated with normal human blood and in various human conditions. Schilling-Torgau [1] also does not think that these bodies are parasites but that they represent structures occasionally present within the erythrocytes of man and animals. Wenyon [2] in writing of these bodies described by Seidelin says even if one accepts the statement that the bodies are present in cases of yellow fever and absent in other conditions the author has yet to prove that they are parasitic and if so are the etiological factor in the production of yellow fever. Wenyon and Low [3] also believe they have found the same bodies in normal guinea pigs' red blood-corpuscles.

[1] Schilling-Torgau: Beiheft 1, Archiv. f. Schiffs- u. Tropenhyg., Leipz., 1912, xvi, 87. Archiv. f. Schiffs- u. Tropenhyg., Leipz., 1912, xvi, 373.

[2] Wenyon: Tropical Diseases Bull., Lond., 1912–1913, I, 249.

[3] Wenyon and Low: J. Trop. Med. and Hyg., Lond., 1914, xvii, No. 24, 369.

IX

LINGUATULIDA

Crocodiles are numerous in the rivers near Guayaquil, and a number of them were obtained from the Guayas river varying from about four to ten and one-half feet in length. (Plate xlvii.) The specimens obtained proved to be of the same species, and one preserved and brought to this city has been identified by Dr. Thomas Barbour, Curator of Oceanica and instructor upon Poisonous Reptiles in this School, as *Crocodilus americanus* (Laurenti). According to Barbour this species has a wide range of distribution, having been found in south Florida, the Greater Antilles, central and northern South America. It reaches the southern limit of its distribution on the west coast of South America in the Guayas river area. In three of four of the crocodiles examined a linguatulida of the genus Porocephalus was found in the lungs. (Plate xlviii.) In one instance the lung showed at the apex a considerable abnormal fibrosis. A nodular mass was found measuring about five millimeters in diameter, which on section consisted of dry grayish cheesy material. On cutting in various directions through the lung, elongated and slightly flat parasites were found varying from 0.5 to 2.5 centimeters in length usually, having in general a pale pink color, with bright red and dark brown areas visible in their interior. Some of these were encysted or situated in the lung substance proper, and none were found free in the bronchi or in the vessels. The lungs of two other crocodiles were placed over night in formalin. The following morning decomposition had already commenced. Upon dissection of all of the lungs a large number of linguatulidae were found. Some of these were free in the bronchi and in the large air chambers. Possibly some of them had migrated after the death of the host. A number of the parasites were somewhat macerated and had lost their pink color. The parasites were

referred to William M. Wheeler, Professor of Economic Entomology in this University and in charge of the instruction in Tropical Entomology in this School, who reports that while the species is very closely related to Diesing's *Porocephalus gracilis*, it is evidently a new and undescribed one. Professor Wheeler's account of this parasite is found in the Appendix on page 207; to it he has given the name of *Porocephalus crocodili*.

Fig. 1. — Crocodile (Crocodilus americanus) shot in the Guayas River.

Fig. 2. — Daule River. Favorite haunt of crocodiles.

Plate XLVII. — Ecuador.

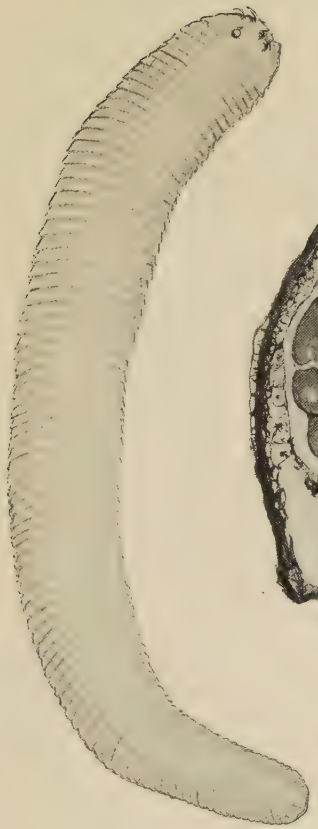


Fig. 1. — Adult Female of Linguatulida (*Porocephalus crocodili* sp. nov.). About ×21.

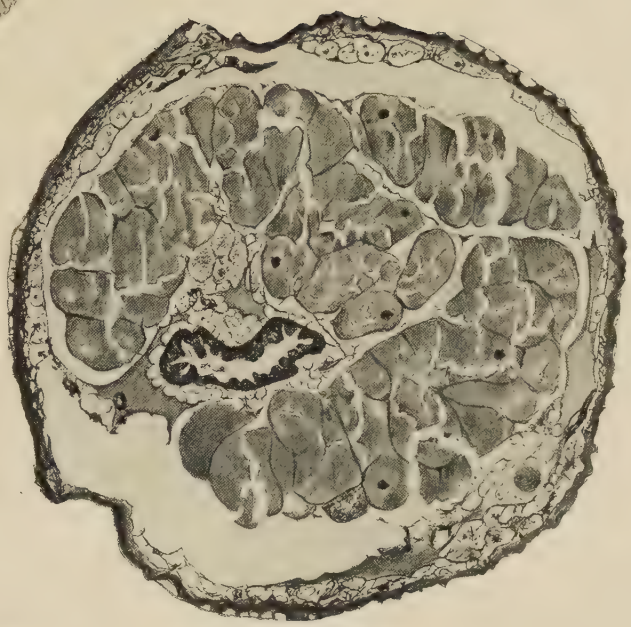


Fig. 2. — Cross-section of Adult (*Porocephalus crocodili* sp. nov.). × 114.

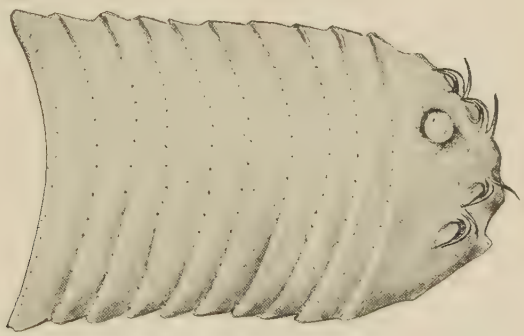


Fig. 3. — Greater Magnification of Head. Showing mouth and hooklets. About × 61.

Plate XLVIII. — Linguatulida from lung of crocodile.

X

ACKNOWLEDGMENTS

In connection with the investigations outlined in this report we wish to express publicly our thanks, particularly to the Corporation of Harvard University for generously providing funds for the maintenance of the expedition and to the Dean of the Harvard Medical School and the Dean of the Graduate School of Medicine for their interest and assistance in the organization of the expedition; to the United Fruit Company of Boston for generously furnishing us with transportation from New York to Colon and return; to the Royal Mail Steamship Company and the Pacific Steamship Navigation Company for furnishing us free transportation from Balboa to Guayaquil and from Callao to Balboa, and to the Panama Railroad Company for courtesy in regard to handling our baggage across the Isthmus. We also wish to express particularly our gratitude to Mr. J. W. Blaisdell, assistant manager of the Central Railroad Company of Peru, who very generously on several occasions furnished us with special railway facilities in our studies carried on between Lima and Oroya, and to Dr. W. F. Bailey, Cerro de Pasco Railway, for many kindnesses during our expedition from Oroya to Cerro de Pasco. To the following physicians and officials we wish to express our grateful acknowledgment for many kindnesses and for assisting us in obtaining material in connection with our studies: Drs. Deeks, Perry, Caldwell, Darling and James in Panama; Dr. Parker, Consul General Baker and Dr. Pareja and other officials of the Board of Health in Guayaquil; President Billinghurst of Peru, the American minister, Mr. Henry Clay Howard, the introductor of ministers, Mr. Cisneros, and Drs. Odriozola, Arce, Graña, Barton, Hercelles and Matto in Lima. Dr. Pareja particularly assisted us in obtaining pathological material in relation to yellow fever and Drs. Odriozola, Arce, Graña and Barton in finding cases of verruga peruviana. Dr. Hercelles supplied us with a number of tissues from Oroya fever cases. We also wish to express our great appreciation of the very valuable assistance rendered us by Dr. J. C. Tello, Director of the Ethnologic Museum, Lima, in facilitating our obtaining material and in assisting us in making arrangements in regard to some of our investigations. Without Dr. Tello's assistance some of our investigations would probably not have been brought to as successful an issue as they were. Finally, we wish to express our deep appreciation of the efficient services rendered by Miss Nora Dwyer, who accompanied us as secretary on the expedition.

The drawings of the microscopical preparations accompanying the report were made by Miss E. R. Piotti, and the photomicrographs by Mr. L. Brown. Miss C. M. Casassa has been of much assistance in reading the proof, and we are greatly indebted to Mr. C. C. Lane for having seen the report through the Harvard University Press.

APPENDIX

APPENDIX

I. A NEW LINGUATULID FROM ECUADOR

By William Morton Wheeler
Professor of Economic Entomology

Among the forms described by Diesing in his well-known monograph of the Linguatulids [1] are two South American species called respectively *Pentastoma gracile* and *P. furcocercum*, which differ from the other species in having the hamuli, or hooks surrounding the mouth geminate, or double. These species were later transferred to the genus *Porocephalus* Humboldt when this name was substituted for *Pentastoma* Rudolphi and restricted to species having a cylindrical body, without lateral diverticula of the body-cavity in each annulus. The two species described by Diesing, however, should probably constitute a distinct genus, with *P. gracile* Diesing as the type. A third undescribed species belonging to this same group and recently taken by the Harvard Expedition to South America from the lungs of a crocodile (*Crocodilus americanus*) captured in the Guayas river, at Guayaquil, Ecuador, were referred to me for identification. The specimens were mostly small and immature, but they included an adult male and female and were sufficiently well preserved to enable me to draw up the following specific diagnosis:

Porocephalus crocodili sp. nov.

The specimens vary from 3–25 mm. in length. The body is pale yellow, slender, cylindrical, blunt at the ends, usually slightly broader anteriorly, with the posterior half or third curved ventrally. The annuli are numerous, 105 in one male specimen measuring 25 mm., and 75 in a specimen measuring only 5 mm. They are very narrow, but quite sharply marked off from one another both in large and small specimens, except at the extreme posterior end. Anteriorly the head bears a pair of small rounded sensory papillae and a second pair of similar but less prominent structures further apart on its dorsolateral surface. The pores on the borders of the annular folds are very small, and rather indistinct. The mouth opening is large and elliptical or more rarely circular, as shown in Plate xlviii, Figs. 1 and 3. It is surrounded by four pairs of double chitinous hooks which arise from elongated slits with welt-like margins. In each pair the posterior hook is strongly curved, the anterior distinctly more attenuate and

[1] Versuch einer Monographie der Gattung Pentastoma, Ann. Wien. Mus. Naturgesch. I, 1836, pp. 1–32, pls. 1–4.

more nearly straight. In the male the genital papilla, which is situated in the mid-ventral line on the second annulus behind the mouth, is small but projecting.

The specimens when found were partly free in the bronchial cavities and partly encapsuled in the lung tissues of the crocodile. They were preserved in formalin.

P. crocodili is evidently very closely related to Diesing's *P. gracilis*, but this author's figures show both hooks of a pair to be equally developed and strongly curved, which is certainly not the case in *P. crocodili*. In the latter, moreover, the hooks are much smaller, less projecting and less heavily chitinized. Diesing based his species on a large number of specimens collected by Natterer at Cuyaba, Caicara and Villa Maria, Brazil. They were found either free in the body cavity or encapsuled in the viscera and mesenteries of lizards, snakes and fish. Among the lizards cited as hosts are two species of *Podinema*, among the snakes, species of *Elaps*, *Coluber*, *Bothrops*, *Pseuderys*, *Tropidonotus* and *Eunectes*, and among the fish, species of *Lobotes*, *Silurus*, *Piara*, *Pimelodes*, *Salmo*, *Serrosalmo*, *Clupea*, *Erythrinus*, *Symbranchus*, *Gymnotus*, *Sternarchus* and *Raja*. The other species of *Porocephalus* with double hooks (*P. furcocercum*) was also taken by Natterer at Cuyaba, in the body cavity, lungs and mesentery of snakes (*Coluber* and *Spilotes*) and *Amphisbæna flavescens*. This Linguatulid is readily distinguished from *gracilis* and *crocodili* by the peculiarly bifurcate posterior end of the body and the three papillae around the base of each pair of hooks. In this case the hooks are more like those of *P. crocodili*, as Diesing says: " Der obere ist fast gerade und kürzer, der untere länger, mehr nach innen gebogen." Had this been the case in *gracilis* Diesing could hardly have failed to call attention to the difference in his description or to show it in his figures.

II. SOME FLIES OF THE FAMILY PHORIDÆ OBTAINED BY THE EXPEDITION, WITH NOTES ON A SPECIES POSSIBLY ASSOCIATED WITH EXTERNAL MYIASIS IN MAN

By Charles T. Brues
Assistant Professor of Economic Entomology

Aphiochæta ferruginea Brunetti

While in Buenaventura, Colombia, on May 14, some scrapings were taken from the skin of a negro affected with caraate, and placed in two test-tubes containing an agar medium. These were well plugged with cotton, fastened in place with paraffin and placed in a wire basket together with a number of similar tubes inoculated from other sources. On examining the tubes after reaching Guayaquil on May 18, a number of minute Dipterous larvae were found feeding on the surface of the agar in the two tubes mentioned, although there were none present in any of the other tubes which had been kept in the same basket. From this it appeared that the larvae must have been derived from the skin of the negro rather than have worked into the tube later, as all the tubes contained the same agar medium. The larvae developed very slowly, but finally pupated during the first week of June and began to emerge as adult flies between June 26 and July 1. They then proved to be a species of Phoridæ belonging to the genus Aphiochæta.

Although the evidence is not complete, that either eggs or freshly hatched larvae were present on the skin of the negro when the scrapings were made, it seems very probable that such was the case. Even so, it is of course doubtful if this is a regular habit of this species. However, the material was obtained from the back of the patient and the skin at this point was exceedingly rough and scaly, so that eggs or larvae of such minute size would readily have escaped notice at the time the tube was inoculated.

There is some doubt concerning the identity of this species and on this account there is given a description based on the specimens reared from the larvae obtained at Buenaventura. *Aphiochæta ferruginea* was first described by Brunetti from India (Rec. Indian Museum, vol. 7, p. 84 (1912)), but has been recorded by Austen from West Africa, the West Indies and Central America. Cotypes of Brunetti's species have been examined by the writer and also what appears to be the same species from Abyssinia. There is considerable variation however in the several lots and more than one species may be concerned.

The Indian *A. ferruginea* has been found to cause myiasis of the intestines and was long ago reported on by Baker in the Proceedings of the Burma Branch of the British Medical Association for 1891, pp. 11–16 and 28–29. In British Honduras, Heuser has bred the Central American form

from larvae extracted from an Indian's foot and says that the species is abundant about the mangrove swamps at Belize where it occurs about decomposing shell-fish.[1] The specimens from Abyssinia were reared from dead and imperfectly dried beetles, and Brunetti, Smith and Austen have reared adults from decaying insects and putrid meat.

APHIOCHÆTA FERRUGINEA Brunetti (Plate XXXIII, fig. 3)

♂ ♀ Length 1.3–1.6 mm. Brownish-yellow; front fuscous, black about the ocelli; thorax above brownish; abdomen above piceous basally and black apically, the base of the second segment and a median spot on the third and fourth segments orange-yellow; hind femora tipped with black; pleurae and venter basally, pale testaceous. Wings hyaline, venation pale fuscous. Front about as broad as high, with four proclinate bristles of nearly equal size, the lower pair half as far apart as the upper pair, the latter in a line with the lower reclinate bristle of the next row and slightly closer to it than to the median line. Lateral reclinate bristle of this row high up and but little nearer the eye than the median bristle. Four reclinate bristles of next row above equidistant, forming a nearly straight line; bristles of ocellar row normal, no larger than the others. Ocellar tubercle and median frontal groove present; surface of front subshining. Cheeks each with two equal, downwardly-directed macrochætæ. Antennae small, rounded, fulvous, with a densely pubescent arista that is distinctly longer than the head-height. Palpi pale yellow, moderately large, with five or six large macrochaetae on apical third below, bare basally. Scutellum with four bristles, the lateral pair scarcely smaller than the others; mesonotum with one pair of widely separated dorsocentral macrochaetae, but without other bristles along its posterior margin. Mesopleura bare. Abdomen with a small tuft of bristly hairs at each side of the second segment, and with a number of longer conspicuous bristles on the apex of the last two segments, especially in the female. Hypopygium of male small, the anal protuberance pale yellow. Fore legs distinctly thickened, especially the tibiae which are densely clothed with stouter bristles than usual and show a distinct row of about ten setulae on the postero-dorsal surface; middle tibia with a single row of sparsely placed setulae on the postero-dorsal surface; hind tibia with a similarly placed series of 8–10 moderately large setulae. Costal vein reaching the middle of the wing, its cilia rather long and closely placed; first vein entering costa midway between the humeral cross-vein and tip of third vein; second vein entering costa three times as far from tip of first as from tip of third, the cell at the furcation nearly twice as high as long; fourth vein evenly, but not strongly curved for its entire length; fifth and sixth slightly bisinuate; seventh nearly straight, distinct. Halteres pale brownish, strongly darkened on the apical half of the knob.

Among the other Phoridae obtained, one species is new to science and is described below:

APHIOCHÆTA INCARUM sp. nov.

♂ Length 1.3 mm. Thorax pale fuscous, front black, antennae deep yellow with apical half of third joint blackened, palpi pale yellow, pleurae and legs testaceous. Abdomen above piceous, brownish on the fifth and sixth segments, hypopygial appendage pale yellow, halteres pale yellow. Wings hyaline. Front very slightly wider than high; only two supra-antennal bristles, each strong and half as long as the height of the front. Bristles of lowest reclinate row forming a closely placed pair at each lower angle of the front, very close to the eye; bristles of next row forming a line bowed down medially,

[1] Trans. Soc. Trop. Med. & Hyg., Lond., 1910, iii, 230.

its lateral bristles very close to the eye, its medial ones closer to one another than to the lateral bristles. Ocellar tubercle and median frontal line present; surface of front subshining. Antennae rather large, broadly oval, the black on the third joint sharply marked. Palpi with short, weak bristles. Post-ocular cilia moderate, cheeks each with a stout macrochaeta and a series of four small bristles running upwards toward the antenna. Mesonotum shining, finely hairy, with a single pair of dorsocentral macrochaetae placed on the posterior edge of the mesonotum opposite the lateral corners of the scutellum. Scutellum with two large bristles in addition to a lateral microscopic hair. Mesopleura bare, without bristles or hairs above. Abdomen with none of its segments noticeably lengthened or shortened, along the sides with a few conspicuous short bristles, two or three to each segment; on the sixth and seventh segment these are longer and form a series along the posterior margin also. Abdomen opaque, except the seventh segment which is shining and jet-black. Hypopygium shining black. Hind tibiae with distinct, but not strong setulae placed in a single line on the postero-dorsal surface. Wings long and narrow, the costal vein just attaining the middle, its bristles long and sparse, only four between the tips of the first and third veins; first vein ending twice as far from the humeral cross-vein as from the tip of the third; second vein ascending steeply to the costa, forming a cell as long as its own length, entering the costa one-half nearer to the tip of the third vein than to the tip of the first; fourth vein slightly curved, recurved strongly at tip; seventh distinct; all veins rather pale and weak.

One male. Lima, Peru, June, 1913.

This species is related to *A. evarthæ* Malloch[1] from the United States, but differs in the absence of the two lower proclinate bristles on the front and the black, instead of yellow anal protuberance. It does not resemble closely any described South American species.

[1] Proc. U. S. Nat. Mus., 1912, xliii, 472.

III. NOTES ON PERUVIAN MOSQUITOES AND MOSQUITO LITERATURE

By Frederick Knab

Bureau of Entomology, Department of Agriculture, Washington, D. C.

In a recent paper Dr. H. G. Dyar and the writer have indicated the mosquitoes so far known from the Andean and coast regions of Peru.[1] Newstead and Thomas have reported a limited number of species from Iquitos, in the forest region of eastern Peru.[2] Professor C. T. Brues has submitted for my examination a paper containing descriptions of two supposedly new species of Peruvian mosquitoes, published at Lima in 1907. The paper in question is rare and has until now escaped the notice of systematists. Its authors are M. O. Tamayo and C. A. Garcia and it deals with Lake Huacachina and other smaller nearby lakes which are reputed to have therapeutic qualities.[3] A part of the report is taken up with the natural history of the lake, and in that connection two species of mosquitoes are figured and described as new. On page xxxv of the extract "*Anopheles peruvianus* Tamayo" is proposed, and on page xxxvii "*Culex raymondii* Tamayo." The descriptions, although lengthy are unsatisfactory. Large, but rude, figures, on five plates, illustrate both sexes of the imagos, the larvae and pupae of the two species.

The writer is of the opinion that both these proposed new species are synonyms of well known, widely distributed species. This opinion is supported by specimens from Peru.

Anopheles peruvianus is without doubt identical with *A. pseudopunctipennis* Theobald in spite of discrepancies in the description and still more in the figures. From the description and figure of the female the following essential characters may be abstracted: the head has on the vertex a patch of white twisted scales which project in a tuft between the eyes. The palpi are black scaled, with narrow pale rings at the articulations and the last joint yellowish white. Thorax with the median section whitish (gray) and with three characteristic lines. Legs dark, with narrow pale rings at the bases of the tarsal joints, which are entirely covered with very dark scales. Wings with the costa black on the basal two-thirds, followed by a yellowish white spot which originates at the tip of the subcostal vein; this is again followed by black, beyond which the extreme tip is again whitish scaled. The subcostal and first veins show three black spots, the first of

[1] New mosquitoes from Peru. Insecutor Inscitiæ Menstruus, 1914, ii, No. 4, pp. 58–62.

[2] The mosquitoes of the Amazon region. Ann. Trop. Med. & Parasitol., Liverpool, 1910, iv, pp. 141–150, pl. 11.

[3] Las aguas de Huacachina. Informe presentado a la Sociedad Geográfica de Lima. 1907, 4, 63 pp., 12 pls. (Extract from Memória de la Municipalidád de Lima, 1906.)

which is at the basal third, the other two delimit white spots which accompany those on the costa. Two other less conspicuous black spots occur on the third vein and upper branch of the fifth. The one on the third vein lies exactly beneath the costal white spot; the one on the upper branch of the fifth vein is interrupted at the basal cross-vein.

It will be seen that the spots on the wing-veins, as far as indicated, correspond with those of *Anopheles pseudopunctipennis*. Black spots existing in the latter species on the second, fourth and sixth veins, as well as small spots near the base of the fifth vein and the tip of its lower branch are not indicated. Yet it seems certain that these discrepancies are due to omissions or oversight, or, still more likely, damaged condition of the specimens, rather than to any existing differences.

This opinion is substantiated by a specimen declared to be identical with *Anopheles peruvianus* and presented to Professor Brues by Dr. Gastiaburú. As we learn from a footnote on page xxxvii of the treatise of Tamayo and Garcia, Gastiaburú had also considered the species as new and had proposed to describe it as *Anopheles multimaculatis* but in his thesis had suppressed this name. The specimen given to Professor Brues is the one that formed the basis of Gastiaburú's *A. multimaculatis*, later declared to be identical with *A. peruvianus*. It is a male with the spots on the wing-veins somewhat obliterated in the posterior region by loss of scales. However, by careful scrutiny the distribution of black and white scales can be traced and is found to agree perfectly with *pseudopunctipennis*. As to the pale rings stated to exist at the bases of the tarsal joints in *A. peruvianus*, these are merely due to reflection of light and not to any difference in the color of the scales at those points. The ornamentation of the palpi as described for the female *A. peruvianus* corresponds with typical *pseudopunctipennis*.

Anopheles peruvianus is based on specimens from Lake Huacachina. This lake is near Ica, south of Lima and not far from the coast, at an altitude of about 1,200 feet. Tamayo states that *A. peruvianus* is common in different parts of the coast region and that it occurs also in the *montaña*, that is, in the forest region on the eastern slopes of the Andes. The following localities are specifically mentioned. West of the continental divide, Huacachina, San Pedro de Lloc and Lima and its environs; on the eastern slopes of the Andes, Chanchamayo. It should be explained that this last locality is not strictly within the humid forest region, but rather in a partly open transition zone. Gastiaburú's specimen probably came from the vicinity of Lima. Before me are two other specimens of *A. pseudopunctipennis* collected by Townsend in the region about Lima, one at San Bartolomé, altitude about 4,900 feet, the other in Verrugas Canyon, at about 5,500 feet altitude.

When we consider the distribution of *Anopheles pseudopunctipennis*, its occurrence in the Andean and coast regions of Peru appears in no wise

remarkable. It is more especially the common form of Anopheles of the semi-arid American tropics. It occurs from California and south-western Texas southward to northern and north-central Argentina, but within this territory its distribution is governed by local conditions. Collections from Mexico and Central America show that it occurs in the more arid portions and is absent from the humid forest zones. It is common in Panama. In South America it has so far been reported from only a few widely separated localities. The United States National Museum has received specimens through Dr. J. H. Egbert from Santa Marta on the coast of Colombia, a distinctly arid locality. Recently the species has been found in Trinidad (near Port of Spain) by Professor F. W. Urich. The Peruvian localities have been already indicated. Finally, the species has been reported from northern Argentina (Provinces of Jujuy, Salta, Tucumán and Santiago del Estero), again a semi-arid region.

Turning now to the second mosquito described by Tamayo and Garcia, *Culex raymondii*, its identification must be purely inferential. Neither the description nor the figures offer details that could lead to an exact diagnosis. The figure of the larva shows by the somewhat elongate breathing tube with several hair tufts, together with the large antennæ with large, outwardly situated tuft, that the insect belongs to Culex in the restricted sense; but beyond this the crude figure does not permit one to go. The female is described as having a dark proboscis and palpi, black erect forked scales on the occiput, the recumbent scales yellowish white. Mesonotum without ornamentation. Abdomen dorsally blackish, the segments with basal white bands; venter pale. Legs dark, the tarsi with whitish basal bands, most distinct on the first joint. Claws simple. Wings unspotted, the scales uniformly dark colored. Total length (including proboscis) 8.5 mm. The larvæ are said to occur in unused wells in the vicinity of Lake Huacachina.

There is every probability that *Culex raymondii* is nothing but the widely distributed, semi-domestic *Culex quinquefasciatus* (= *fatigans*). The description fits it very well, as far as it goes, with the exception of the ringed tarsi. In the figures of the two sexes the tarsi show no rings, and it is safe to assume that they are as little in evidence in this form as in the *Anopheles peruvianus* already discussed. *Culex raymondii* is said to occur in Lima and its outskirts, and this again points to our widely spread species of domestic habits, *Culex quinquefasciatus*, as do the larval habits above indicated.

Following is an annotated list of the mosquitoes now known to occur in Peru. Professor C. H. T. Townsend, until recently entomologist to the Peruvian government, has most liberally placed at my disposal his notes on mosquitoes and mosquito conditions in Peru and I have made free use of them. The Peruvian mosquitoes may be grouped under four headings, as follows:

1. Cosmopolitan species of the tropical and subtropical zones, occurring only in association with man and independent of moisture conditions.

Culex quinquefasciatus Say (*C. fatigans* Auct.)

Reported by Tamayo and Garcia (as *Culex raymondii*) from Lima and vicinity and Huacachina. The following is abstracted from Townsend's notes: "This species has been taken by me from Ancon at sea level to Chosica at 2,800 feet and about the upper limits of the foothill zone. It is the common mosquito of the whole region bordering the coast below an elevation of about 3,000 feet. I found it breeding in the street wells of Ancon, and it is the common mosquito of Lima and vicinity. It is not as abundant at Ancon as *Aëdes calopus*, and seems to be overridden by the latter along the extreme coast line. It is the only mosquito I have observed at Chosica. It is comparatively scarce in individuals both in Lima and in Chosica. A recent epidemic of dengue in Barranco, a suburb of Lima, occurring during the cooler season of 1913, was probably spread by this mosquito."

Aëdes calopus Meigen (*Stegomyia fasciata* Auct.)

The following is from Townsend's notes: "This species extends all the way down the west coast of South America to the Antofagasta region of northern Chile, and doubtless at times much farther to the south. I have found this species at Piura, which is well inland but only two hundred feet or less above the sea. I have found it abundant at Ancon, a beach resort an hour north of Lima by train, where it breeds in the street wells. These wells are largely open, without pumps, or only imperfectly covered, allowing ingress and egress of the mosquitoes. Far within the rain forest region to the east of the Andes, along the navigable upper stretches of the rivers where commerce has introduced it, this species also occurs, notably in the Iquitos region." The species has been reported from Iquitos also by Newstead and Thomas.

2. Coast species breeding in salt or brackish water.

Aëdes epinolus Dyar and Knab

Closely related to *Aëdes tæniorhynchus* and *niger* and perhaps best considered a subspecies. Townsend's observations are as follows: "This is the Peruvian salt-marsh mosquito and conforms in general habits to the typical form in other parts of America. It was found breeding in large numbers during the first week in February (1914) in salt ponds immediately back of the beach at Ventanillas, an uninhabited point about nine miles south of Ancon and about half way between the latter and Callao. The ponds in question vary from small to large and stretch along just inside the beach line for some miles, being formed by inroads of the sea during unusually rough weather. This species reached Ancon in great swarms during the last week or so of January, 1914, and the municipality called upon the writer to make an investigation of the plague. On February 3 the adults were still to be found in some numbers in Ancon, though rapidly disappearing, while it was determined that the species was not breeding there. On February 4 the ponds at Ventanillas were visited. Small ponds showed numerous large nearly full-grown larvae, without either small larvae or pupae. Large ponds showed no signs of larvae. Rims of cast pupal skins just above the waterline, stuck to the hard gravel, fringed the ponds, having been left to dry as the water evaporated to a lower level. The adults were present in swarms and very blood-thirsty. The only blood supply present in this immediate region of the coast is that furnished by the seafowl that continually haunt the coast line. On January 11, 1914, the sea was unusually

heavy along this region of the coast. It evidently overrode the high natural breakwater of smooth pebbles that forms the beach at Ventanillas, thus producing a great breeding area for this mosquito. As a result immense swarms migrated from these ponds a week or two later, and these reached Ancon in force to the great dismay of the inhabitants."

3. Species of the dry regions of the mountains and coast.

Anopheles pseudopunctipennis Theobald

(Synonyms: *franciscanus* McCracken, *tucumanus* Lahille, *argentinus* Brèthes, *peruvianus* Tamayo, *multimaculatis* Gastaburú)

The known distribution has been already indicated in the previous discussion. Townsend thinks that in Peru the species probably ranges considerably above 8,000 feet. Its occurrence must be more or less local, depending, as it does, upon the presence of suitable breeding places, such as isolated pools in stream-beds, water-holes, abandoned wells, etc.

Anopheles species, near *maculipes* Theobald

A single female specimen in rather poor condition presented to Dr. Brues by Dr. Gastiaburú, along with the specimen of *A. pseudopunctipennis* already discussed. The locality is uncertain, but most probably not remote from Lima. The specimen resembles *A. maculipes* and related species in the coloration characters and in the shape of the wing-scales, but differs in numerous details. It probably represents an undescribed species, but it seems inadvisable to found a species in a difficult group on a single poor specimen of uncertain origin.

Phalangomyia debilis Dyar and Knab

Matucana (Brues).

4. Species of the humid forest region east of the Andes.

Anopheles boliviensis Theobald

(Synonyms: *lutzii* Theobald, not Cruz; *cruzi* Dyar and Knab)

Described twice by Theobald, first from southern Brazil and then from Songo, Bolivia. The latter locality is presumably on the eastern side of the Andes and within the forest region. This species breeds almost exclusively in water held by the leaves of epiphytic bromeliads and therefore is a typical forest inhabitant. Townsend says: "I took this species in the *montaña* of the province of Jaen, in northern Peru, from the Rio Charape at about 4,500 feet to the lower slopes of Huascaray ridge at about 7,000 to 7,500 feet. It was abundant at both localities and an active biter. Both localities are forested, the Huascaray slopes in patches, the Rio Charape more thickly. Both afford tree holes and epiphytes as breeding places, but do not furnish other standing water, the streams being comparatively swift."

Anopheles tarsimaculatus Goeldi (*albimanus* Newstead and Thomas)

Reported by Newstead and Thomas as the common *Anopheles* at Iquitos. This is a geographic race or subspecies of *A. albimanus*, distinguishable by the difference in the palpal banding. It is the chief malaria transmitter in the Amazon region and throughout most of the Brazil-Guiana forest zone.

Janthinosoma posticata Wiedemann (*musica* Newstead and Thomas)

Reported from Iquitos by Newstead and Thomas. A very widely distributed species east of the Andes.

Aëdes leucomelas Lutz

Townsend took a single female of this species at Hacienda Charape on the Rio Tabaconas, about 3,500 to 4,000 feet, Sept. 18, 1911.

Mansonia titillans Walker

Iquitos (Newstead and Thomas). Widely distributed through the American moist tropics, but owing to the peculiar larval habits local in occurrence. The larvae inhabit permanent waters and live attached to the roots of certain aquatic plants from which they obtain the needed air. The female is an aggressive blood-sucker and when abundant a serious pest.

Mansonia pseudotitillans Theobald

A single specimen from Iquitos doubtfully so identified by Newstead and Thomas.

Culex chrysothorax Newstead and Thomas

(*Neomelaniconion chrysothorax* Newstead and Thomas, not *Melanoconion chrysothorax* Peryassú)

Described from Iquitos, Peru, and Manáos, Brazil. The specific name is preoccupied by the species of Peryassú, as the two species are congeneric and belong in *Culex*. I refrain from proposing a new name, as the species may be identical with others already described.

Limatus durhami Theobald

Reported from Iquitos by Newstead and Thomas.

Sabethes species

Townsend has the following note on a species of this genus, the members of which are strictly forest insects. " I took two specimens on February 12, 1910, at Yahuarmayo on the Rio Inambari, about 1,700 feet, in the southern *montaña* of Peru. The species is of a brilliant metallic coloring, with feathered legs. It was determined by Dr. A. Lutz as this genus, with the note: ' Very much like a species I brought from the Amazons region.' "

IV. HETEROPTERA FROM THE WEST COAST OF SOUTH AMERICA

By J. R. de la Torre Bueno

The few species collected in Ecuador and Peru in 1913, by the expedition from the School of Tropical Medicine of the Harvard Medical School have been submitted to me for determination. All, except one, were taken at Guayaquil in Ecuador; nine of the thirteen Ecuadorian species are new records for that country, and three considerably extend the range of North and Central American species. The Peruvian species, *Nysius spurcus* (Stal) is also a new record and greatly enlarges its area of dispersal. On the whole, small as is the number of species listed, it contributes materially to our knowledge of distribution.

The arrangement of the Cimicids (Pentatomids) is according to Kirkaldy; the remaining families are arranged according to Lethierry and Severin's Catalogue and to Bergroth's supplements thereto.

Thyanta antigüensis Westw.

There is one specimen only of this small species, which has seemingly not been heretofore recorded from South America; the Southwestern United States, Mexico, Central America and the Antilles being the known range.

Symphylus deplanatus H. S.

This species has not been recorded heretofore from further south than Panama. This would seem to be its first reported appearance on the Pacific coast of South America, where one specimen was secured.

Pachylis laticornis Fabr.

Only one specimen of this wide-spread and common form was obtained. It has been recognized heretofore from Costa Rica to the Argentine Republic, including Ecuador, in the Transandean region, but not from the Pacific side.

Capaneus obscuratus Mont.

One typical ♀ specimen was collected at Guayaquil, the type locality.

Machtima crucigera Fabr.

A ♂ and a ♀ are present, of this unmistakable species, which would seem to be known from Brazil only, so far as published records available are concerned. This is a new record and remarkably extends its range.

Hypselonotus lineatus Stål

The ten specimens sent are the first to be known from the Pacific coast of South America. The species, described from Mexico, has also been

secured in Honduras, on the Atlantic (or rather, Gulf) side of Central America.

Harmostes reflexulus Fabr.

There is one typical specimen of this species in the collection, first record from Ecuador, although previously known from such widely separated lands as Mexico and the Argentine Republic, but from none of the intervening countries.

Rhopalus rufescens Spinola

This species hitherto known only from Chile, whence it was first described, is represented by one specimen from Guayaquil.

Rhopalus sidae Fabr.

There are two Guayaquil specimens of this common form in the collection, a first record, although it is said to be distributed from Mexico to Argentine Republic.

Dysdercus ruficollis Linne

This abundant and wide-spread form is represented by nine specimens. It is known from all South America (except Peru and Chile) and from Mexico as well.

Nysius spurcus Stål

The one specimen present from San Bartolomé represents the Hemipterous fauna of Peru. It is of interest, as heretofore it has been known only from Mexico, Honduras and Brazil.

Melanolestes morio Er.

This darkling Reduviid is represented by one specimen from Guayaquil, seemingly the first Ecuadorian record. Its previous records are from Colombia, the Guianas and Brazil.

Zelus (*Diplodus*) sp.

In the collection there are 7 specimens of an undetermined species from Guayaquil. This genus is sadly in need of a careful revision, especially of the tropical American forms.

Belostoma asiaticum Mayr.

Nine specimens are present. Apparently this species heretofore was unknown from Ecuador, although recorded from Peru and Chile on the Pacific coast, as well as from Brazil, Mexico and Argentine Republic.

V. LIST OF THE BEES OBTAINED BY THE EXPEDITION

The present list is taken from a paper by Professor T. D. A. Cockerell,[1] who kindly examined all of the bees that were collected.

Xylocopa brasilianorum, subsp. *bruesi* Ckll. San Bartolomé.
Xylocopa transitoria Perez. Guayaquil.
Eulaema bruesi Ckll. Guayaquil.
Bombus coccineus Friese. Matucana.
Melipona mimetica Ckll. Guayaquil.
Trigona leucogastra Ckll. Guayaquil.
Megachile pyrrhogastra Ckll. Guayaquil.
Megachile philinca Ckll. Guayaquil.
Megachile garleppi Friese. San Bartolomé.
Megachile ecuadoria Friese. Matucana.
Coelioxya haematura Ckll. Guayaquil.
Coelioxys leucochrysea Ckll. Guayaquil.
Coelioxys rufibasis Ckll. Guayaquil.
Coelioxys tumerifora Ckll. San Bartolomé.
Hypanthidium ecuadorium Friese. Guayaquil.
Anthidium 22-punctatum Friese. Guayaquil.
Anthidium matucanense Ckll. Matucana.
Triepeolus megadelphus Ckll. Guayaquil.
Centris nitida, subsp. *geminata* Ckll. Guayaquil.
Melitoma euglossoides Lep. & Serv. Guayaquil.
Xenoglossa citrullina Ckll. Guayaquil.
Florilegus pavoninus Ckll. Guayaquil.
Florilegus purpurascens Ckll. Guayaquil.
Melissodes ecuadoria Bertoni & Schrottky. Guayaquil.
Leptometria pacifica Ckll. Guayaquil.
Chalepogenus alfkeni Ckll. Guayaquil.
Exomalopsis zexmeniae Ckll. San Bartolomé.
Exomalopsis bruesi Ckll. Guayaquil; San Bartolomé.
Agapostemon nasutus Smith. San Bartolomé.
Augochlora binghami Ckll. Guayaquil.
Augochlora metallica Fabr. Guayaquil.
Augochlora thalia Smith. Guayaquil.
Augochlora cladopyga Ckll. Guayaquil.
Augochlora matucanensis Ckll. Matucana.
Lonchopria inca Ckll. Matucana.
Colletes miminca Ckll. Matucana.

[1] J. New York Entom. Soc., 1914, xxii, pp. 306–328.

616.92

616.92
qH261r

Harvard university.Med.
school. Dept.of tropical me
Report of first expedi
tion to S.America.

DARTMOUTH COLLEGE
3 3311 02325 0584